5-1989

Influence of Processing Parameters on Nutrient Recovery During Ultrafiltration of Milk and Meltability of Pasteurized Process Cheese Food made from the Retentate

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INFLUENCE OF PROCESSING PARAMETERS ON NUTRIENT RECOVERY DURING ULTRAFILTRATION OF MILK AND MELTABILITY OF PASTEURIZED PROCESS CHEESE FOOD MADE FROM THE RETENTATE

by

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A dissertation submitted in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

UTAH STATE UNIVERSITY

Logan, Utah

1989

ACKNOWLEDGEMENTS

I would like to thank Kraft and the Dairy Research Advisory Board for funding this research. I am especially grateful to my advisor, Dr. C. Anthon Ernstrom, for his support and encouragement. Special thanks go to committee members, Dr. Rodney Brown, Dr. Gary Richardson, Dr. Paul Savello, Dr. Elizabeth Boeker, and Dr. Don Sisson for advice and helpful suggestions. Fellow workers, Eric Bastian, Marie Walsh, Dr. Nana Yiadom-Farkye, Rachaneeporn Chaistitwanich, Max Torres, and Ethel Maynes all contributed extensively to data collection and planning of the experiments. I want to thank my husband, Mark, for his endless love and support throughout this project.

Susan Kay Fortier Collinge

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ABSTRACT

Influence of Processing Parameters on Nutrient Recovery During Ultrafiltration of Milk and Meltability of Pasteurized Process Cheese Food made from the Retentate

By

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Utah State University, 1989

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Three batches of milk were ultrafiltered to 60, 65, or 70% volume reduction before diafiltration. Starting diafiltration at 70% volume reduction took less time and water without affecting nutrient recovery.

Whole milk was heated to 60, 72, and 82°C for 16 s. Milk representing each heat treatment was divided into three batches, one unacidified (pH 6.6), the others acidified to pH 6.2 and 5.8. The milk was ultrafiltered, diafiltered, and concentrated to 5× (80% volume reduction). Retentate was inoculated with .5% lactic culture and incubated at 28°C to pH 5.1. Each lot of fermented retentate was evaporated under 76 kPa vacuum until moisture was reduced to 35-38%, then made into pasteurized process cheese food by cooking to 82°C. The final product contained 43-44% moisture, 24-28% fat, 1.7% salt, and 2.5% sodium citrate. Fat and protein recovery were not affected by heat treatment or pH adjustment of the milk. Recovery of calcium, phosphorus, and riboflavin were significantly reduced following acidification of milk. Riboflavin recovery was higher when milk was preheated to 60°C as opposed to 72 or 82°C.

Effect of cooking temperature on meltability of process cheese food was evaluated by repeating the above experiment at three cooking temperatures, 70, 76, or 81°C. Cooking temperature significantly affected meltability. Cheese cooked to 70°C melted best for all treatments. At all cooking temperatures, cheese from unacidified milk (pH 6.6) had greater meltability than cheese from milk acidified to pH 5.8 or 6.2. Cooking temperature had a greater effect on meltability of process cheese food made from ultrafiltered retentate than calcium content. Preheating milk before ultrafiltration did not significantly affect meltability of pasteurized process cheese food.

Meltability of pasteurized process cheese food was best when made from retentate heated (following ultrafiltration) to 61°C for 16 s and poorest when retentate was heated to 72 or 83°C.

During ultrafiltration without diafiltration, amino acid analysis was on samples taken at 0, 20, 40, 60, and 80% volume reduction. There were no differences in amino acid composition (g/100 g protein) between milk and 5× retentate.

Soluble nitrogen at pH 4.6 in pasteurized process cheese food was an approximate measure of undenatured whey protein. As processing temperature increased from 66 to 82°C, undenatured whey protein decreased. Decrease in meltability due to increased processing temperature was related to denaturation of whey protein.

Process cheese food made from blends of UF curd and Cheddar cheese had acceptable meltability with up to 66% UF curd when the final processing temperature was 68°C.

Milk with high bacterial numbers (7.8×10^6 CFU/ml) was heated to 72°C for 16 s, acidified to pH 5.8 and ultrafiltered to a 5× concentration. Ultrafiltration proceeded normally and no processing difficulties were encountered.

(123 pages)

INTRODUCTION

Ultrafiltration (UF) is a process that allows passage of small molecules like water and salts through a semipermeable membrane while retaining larger constituents such as fat and protein. Because most of the whey protein is retained by UF, it has potential for increased yield in cheese making and increased utilization of nutritious milk proteins.

Interest in using UF as a first step in cheese making has required accumulation of nutritional information about retentate to ensure that products made from it are equivalent to those made by the traditional process. Nutrient retention during UF could be affected by high bacterial numbers in the milk, varying the beginning point of diafiltration, and heat treatment of milk.

By using a combination of ultrafiltration and vacuum evaporation, a product similar in chemical composition to Cheddar cheese can be produced (29). This product, called "cheese curd for processing" or "UF curd", can be obtained with a 16-18% increase in yield over traditional Cheddar cheese processes. Such cheese curd can then be combined with other cheeses to make pasteurized process cheese products. This process is currently in use by one of the largest manufacturers of pasteurized process cheese.

It was noted by Ernstrom et al. (29) and Sood and Kosikowski (80) that process cheese made with 80% UF cheese curd has poor melting quality. To understand this difference in meltability, the effect of added whey protein (72) and increased calcium in process cheese was studied (2, 28).

The purpose of this study was to evaluate factors affecting retention of milk nutrients during UF. Processing parameters were varied to consider bacterial quality, beginning point of diafiltration, acidification, and heat treatment of milk. The cooking temperature of process cheese food made from UF retentate was evaluated with respect to meltability. Undenatured whey protein was measured following cooking of process

cheese food to temperatures between 66 and 82°C to explain how whey protein and heat affect meltability of process cheese food.

The specific objectives were:

1. Determine if amino acid composition of milk changes during ultrafiltration.
2. Compare nutrient composition of process cheese food made from UF curd and traditional Cheddar cheese.
3. Study nutrient recovery and retention during UF of milk with high initial bacterial numbers.
4. Evaluate nutrient recovery and overall process efficiency while varying the beginning point of diafiltration.
5. Study the effect of heat treatment and acidification of milk before UF on nutrient recovery and retention.
6. Investigate the effect of heat treatment and acidification of milk before UF on meltability of process cheese food made from UF curd.
7. Study the effect of processing temperature on whey protein denaturation in process cheese food made from UF curd.
8. Blend UF curd with Cheddar cheese to achieve optimum meltability in process cheese food.
9. Determine the mechanism for loss of meltability of process cheese food made from UF curd with increased processing temperature.

LITERATURE REVIEW

Nutrient Retention and Recovery During Ultrafiltration

Ultrafiltration of milk, as a preliminary step in cheese making, is an important area of dairy research. The concentrate, or retentate has been used to make Cheddar cheese (11, 33, 82) process cheese (29, 80), cream cheese (15), cottage cheese (30, 55), Camembert and goat's milk cheese (56), and mozzarella cheese (16). During ultrafiltration (UF), milk is separated into two phases; water and soluble nutrients pass through a membrane as permeate while protein, fat and associated nutrients are concentrated as retentate. To assess nutrient quality of products made by UF, it is important to know what portions of milk nutrients are retained and what pass through the membrane. During a batch UF process, milk is held at 50-55°C and can be exposed to light. Both heat and light could affect nutrient quality of retentate. While developing processing techniques, many researchers measured protein, fat, and lactose in retentate (29, 67, 80, 82, 90).

Green and co-workers (34) performed an extensive nutrient analysis of non-diafiltered retentate to determine recovery of protein, fat, lactose, nicotinic acid, biotin, vitamin B_6, pantothenic acid, riboflavin, vitamin B_{12}, folic acid, Ca, Mg, Zn, Fe, Cu, P, and citrate. Fat, protein and vitamin B_{12} are completely retained by the membrane. Water soluble vitamins and most minerals are only partly retained. Most minerals are concentrated less when lactic acid or sodium citrate are added. Acidification causes a release of calcium, phosphorus and some other ions from the casein micelle.

Green et al. (34) found that casein micelle size is not changed by ultrafiltration. Lonergan (53) did not see any changes in micelle size or distribution of casein between micelles and serum during normal ultrafiltration and diafiltration. Calcium and phosphorus content of micelles is not changed.

Amino acid analysis of milk and retentates could be used to determine if the nutritional quality of protein is affected by UF. However, no information has appeared in the literature on this point. Bastian (7) studied nutrient composition of retentate and permeate during UF with and without diafiltration and following acidification with HCl. Results of this study were similar to those of Green et al. (34), except some whey protein was found in the permeate. Using PAGE, Bastian (7) identified β-lactoglobulin and α-lactalbumin in the permeate. Greater loss of protein may have occurred because he used a 10,000 molecular weight (MW) cut off membrane whereas Green et al. (34) used a 3,000 MW cut off membrane. As the smallest of the major milk proteins, β-lactoglobulin and α-lactalbumin (molecular weights of 18,000 and 14,000) are most likely to pass a membrane with a 10,000 MW cut off. To date, little information is available on nutrient retention of preacidified, diafiltered retentate and process cheese food made from these retentates.

Heat Treatment Before Ultrafiltration

Heat treatment of milk can cause partial denaturation of whey proteins (76) and may affect protein retention during partitioning by ultrafiltration. Some α-lactalbumin and β-lactoglobulin pass the ultrafiltration membrane (7,67), however losses may be reduced by partial denaturation or complexing with caseins during heating. Extraction procedures for analysis of water soluble vitamins such as riboflavin and vitamin B_{12} use heat to release the vitamins from proteins (13, 54). Heat treatment of milk before UF could cause loss of these vitamins during UF and diafiltration. Preheating milk improves the yield of mozzarella cheese made by direct acidification (76). Yield is improved because some whey protein is denatured or complexed with casein and trapped in the curd.

Process Cheese from Retentate

Using ultrafiltration and vacuum evaporation, Ernstrom et. al. (29) made cheese curd (UF curd) with 16-18% higher yield compared to conventional methods. Using

80% UF curd with 20% aged Cheddar cheese, the process cheese had good flavor but was described as "excessively stiff". Sood and Kosikowski (80) developed a method for making process cheese from Cheddar cheese and skimmilk retentate. With up to 40% retentate, the process cheese was comparable to commercial process cheese. When 80% retentate was used, texture was judged defective and flavor was bland. To improve flavor and texture, process cheese was made with retentates modified by treatment with fungal protease and lipase. With enzyme modified retentate, process cheese of acceptable flavor could be made with up to 60% retentate.

Sood and Kosikowski (80) found a decrease in melting index from 79 to 14.3% when retentate solids are increased from 0 to 80% in process cheese. Cheeses were cooked to 75°C in 10 min. Total ash was increased from 5.81 to 5.87% with retentate replacement in process cheese. They reported a higher Ca, P, K, and Na content in process cheese made with diafiltered retentate than in cheese made with non-diafiltered UF retentate. Since diafiltration helps remove water soluble nutrients, one would expect Ca, P, K, and Na concentrations to decrease with diafiltration. Sood and Kosikowski's results may have been caused by their use of tap water for diafiltration.

Savello (72) investigated the causes of melting defect in model UF process cheese prepared from casein and milk fat. Since inclusion of extra whey proteins is one of the main differences between traditional Cheddar cheese and UF cheese curd, he studied the effect of added whey protein on meltability of the model system. Adding either undenatured or heat denatured whey protein decreases meltability, although there are no differences between undenatured and heat denatured. However, his cooking temperature of 83°C partially denatured the undenatured whey protein.

Model process cheese containing added whey protein probably decreased in meltability because of the unique functional properties of whey proteins. Whey proteins can form aggregates and gels at temperatures used in cooking process cheese. This may occur in UF process cheese food. Heertje et al. (42) examined process cheese by

transmission electron microscopy. They saw string-like material that resembled the gelation of proteins like ovalbumin, insulin, and lysozyme. These strings may have come from heat-treated whey protein.

Heat Effects on Whey Protein Functionality

The principal whey proteins in milk include β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulins (89). Of the whey proteins, β-lactoglobulin is found in the highest concentration in milk. Milk contains 3.3 g/L β-lactoglobulin, 1.2 g/L α-lactalbumin, .5 g/L immunoglobulin G, and .3 g/L bovine serum albumin (22). Whey proteins are more heat labile than caseins and are of extreme importance in the chemistry of heated milk. With heating, whey proteins form complexes with each other and with casein. Complexes form between β-lactoglobulin and κ-casein with heating (26, 41, 70, 74, 75, 79, 92). Some α-lactalbumin complexes with β-lactoglobulin (6, 47), and both these whey proteins can interact with κ-casein (26). Effects of heating on whey proteins are important in milk pasteurization, ultrafiltration, and cheese, yogurt, cottage cheese, and evaporated milk production.

Chemistry of Whey Proteins During Heating

Reactions that occur during heating of milk include changes in mineral salt balance, protein-protein interactions, and protein-mineral interactions. A principal mechanism for protein-protein interactions is disulfide bonding. The whey proteins all have adequate disulfide and sulfhydryl groups to participate in disulfide interchange and disulfide bonding. A β-lactoglobulin molecule contains five cysteine residues, four involved in disulfide bridges and one free thiol group (22). With heating, this thiol group can react to form intramolecular or intermolecular disulfide bonds with other β-lactoglobulin molecules or other sulfhydryl containing proteins. The most heat labile whey protein is β-lactoglobulin with a denaturation temperature of 70°C (23). The most heat resistant

whey protein, α-lactalbumin, contains eight cysteine residues per molecule, all as disulfide bridges. Bovine serum albumin contains thirty-five cysteine residues per molecule, seventeen as disulfides and one free thiol group. IgG is the most abundant of the immunoglobulins in milk and contains sixty-four cysteine residues per molecule.

Disulfide Formation and Calcium. Milk proteins form intramolecular and intermolecular disulfide bonds when heated. Depending on the type of reaction, heat can contribute to either increased or decreased protein stability. Minerals such as calcium and sodium can affect protein stability and disulfide interaction.

Trautman and Swanson (84) used electrophoresis to show formation of a stable complex between β-lactoglobulin and α-casein in milk heated to 82.2°C for 30 min. During manufacture of evaporated milk, forewarming the milk prevents gelation during sterilization. When p-chloro-mercuribenzoic acid (PCMB), a disulfide blocker, was added at 10^{-4} *M* before forewarming, milk gelled on sterilization (85). Addition of PCMB disrupts the formation of a protein complex involving disulfide bonds, that usually occurs during forewarming. Gelation is prevented during sterilization of evaporated milk because of disulfide formation during forewarming.

Morr and Josephson (61) proposed that disulfide interaction between β-lactoglobulin and κ-casein prevents gross aggregation of denatured whey proteins during heating. Also, whey proteins may form complexes with casein by forming calcium bridges. Protein stability increases when calcium is removed from whey by dialysis with phosphate buffer before heating. Skimmilk dialyzed whey contains more calcium than whey dialyzed with phosphate buffer. N-ethylmaleimide (NEM), a disulfide blocker, causes partial stabilization of whey proteins in the presence of calcium. Without calcium, whey protein is less stable to heat in the presence of NEM. Heat treatment of milk affects soluble Ca and P (44). After heating milk at 80°C for 30 min, concentrations of dissolved Ca and P decreased by 12-18%.

Calcium ions bind to β-lactoglobulin when heated for 30 min at 90°C (92). Precipitation of heated β-lactoglobulin can occur either by reducing the pH to the isoelectric point or by addition of calcium at alkaline pH. Amount of calcium bound depends on the net negative charge of the protein. Aggregation by calcium occurs by a mechanism similar to isoelectric precipitation.

deWit and Klarenbeek (22) studied flocculation of a 1% solution of β-lactoglobulin in the presence of calcium following heating for 15 min at 120°C. As pH is decreased from 7.0 to 6.4 and calcium concentration is increased from 0 to 6 m*M*, stability of denatured β-lactoglobulin decreases rapidly. Aggregation occurs more rapidly in desalted whey. Apparently, formation of calcium phosphate on heating in regular whey leaves less calcium to interact with whey proteins. Higher heat treatment results in less sensitivity to calcium flocculation. Possibly, disulfide formation affects calcium sensitive sites on β-lactoglobulin.

Effects of disulfide formation and calcium during heating of skimmilk were evaluated by adding NEM or ethylenediaminetetraacetic acid (EDTA) before heating at 74°C for 10 s (25). Presence of NEM or EDTA inhibits whey protein denaturation, indicating that disulfides and calcium linkages are part of complexes between whey proteins and casein.

deWit (19) heated β-lactoglobulin in the presence of NEM and increasing amounts of calcium. NEM improves stability of β-lactoglobulin, possibly because of an interrelationship between thiol reactivity and calcium flocculation. Whey protein concentrate flocculates in the presence of calcium, similar to β-lactoglobulin, showing the importance of β-lactoglobulin in heat effects of whey.

Hunziker and Tarassuk (47) found that α-lactalbumin concentration decreases by 84% when heated at 75°C for 30 min in the presence of β-lactoglobulin. When heated alone, α-lactalbumin concentration decreases by 14%. If intermolecular disulfides form protein complexes between β-lactoglobulin and α-lactalbumin, they occur more readily

than intramolecular reactions with α-lactalbumin alone. Aurand et al. (5) observed an interaction between acid casein and serum albumin when heated to 104°C for 15 s.

Hartman and Swanson (41) used polyacrylamide disc electrophoresis to show formation of a complex between κ-casein and β-lactoglobulin caused by heating. When α-lactalbumin or bovine serum albumin is heated with κ-casein, no complex forms. Following heating to 74.5 or 85°C for 30 min, β-lactoglobulin A and B formed a single electrophoretic band. These genetic variants may form intermolecular bonds when heated.

Zittle et al. (93) found that a complex forms when β-lactoglobulin and κ-casein are heated. Before heating, they saw two bands by free boundary electrophoresis but only one following heating at 90°C for 15 min. Clotting time by rennin increases and the curd contains β-lactoglobulin.

When heated, β-lactoglobulin forms complexes involving disulfide bonding with κ-casein and α-lactalbumin. Molecules of β-lactoglobulin can associate by disulfide interactions. Calcium causes flocculation of heated β-lactoglobulin and may promote aggregation between heated proteins.

Mechanism of Aggregation of β-Lactoglobulin by Heat. Heat denaturation of β-lactoglobulin occurs in two steps. The first step forms a tetramer and the second forms larger aggregates. Although researchers agree on this two-stage denaturation, their results conflict about conditions necessary for promoting each stage.

Studies of the kinetics of heat denaturation of β-lactoglobulin show two distinct products (9). The primary product forms by a first order reaction, initiated at temperatures ranging from 65 to 99°C. It results in a four-fold increase in molecular weight. The secondary process, favored at temperatures below 70°C, occurs by a second order reaction after forming the primary product. Above 75°C, rate of formation of the secondary product decreases, and it is totally inhibited at 99°C.

Sawyer (73) investigated two stages of denaturation in β-lactoglobulin. The primary stage forms disulfide bonds by heating at 97.5°C. Secondary stage of denaturation occurs at 75°C and forms larger aggregates. Addition of NEM before heating prevents both the primary and secondary reactions. Mercaptoethanol prevents formation of aggregates in the primary reaction but does not affect formation of the larger aggregates. Both primary and secondary reactions must be initiated by formation of disulfides or disulfide interchange, but the final product of the secondary reaction results from a different, nonspecific aggregation.

deRham and Chanton (18) proposed a slightly different interpretation of the two-step mechanism for denaturation of β-lactoglobulin. True denaturation occurs in the first step and can be blocked by NEM. Extensive polymerization takes place during the second step, which occurs during cooling. deRham and Chanton (18) suggested that calcium aids in formation of aggregates during the second step. Using protein solubility after heating whey as an indicator of denaturation, more denaturation occurs during heating in the presence of added calcium chloride than in demineralized whey. Adding citrate to whey protein concentrates before heating results in less denaturation, indicating that calcium can enhance denaturation.

Elfagm and Wheelock (26) also discussed two types of denaturation of β-lactoglobulin. Following formation of the primary aggregate of four monomers, there is a conversion of the small aggregates to large ones. The large aggregates form more readily at higher temperatures, in contrast to Sawyer's (73) observation of formation at lower temperatures. Complexing between α-lactalbumin and β-lactoglobulin occurs after the small aggregate of β-lactoglobulin forms (27).

Two-stage denaturation of β-lactoglobulin must be considered with an integrated approach. Disulfide interaction is necessary for formation of the tetramers and tetramer formation must occur before polymerization into large aggregates.

Mechanism for Interactions between β–lactoglobulin and κ-casein. Haque et al. (40) studied the mechanism for formation of a complex between β-lactoglobulin and κ-casein. In unheated solutions, β-lactoglobulin forms dimers (A_2) and trimers (A_3). The A_3 complex of β-lactoglobulin reacts with one molecule of κ-casein to form A_4 complexes. The A_4 complexes are apparently stabilized by disulfide bonds since they are disrupted by 2-mercaptoethanol (2-ME) but not by urea.

Solutions of κ-casein and β-lactoglobulin heated at 70°C contain the same A_2, A_3, and A_4 complexes, although the chemical bonding may be slightly different. Unheated A_3 complexes of β-lactoglobulin can be easily dissociated to the A_2 structure. A_3 structures formed by heating are dissociated after treatment with SDS and 2-ME, indicating some covalent association. The authors proposed that A_3 complexes of β-lactoglobulin form hydrophobic bonds with κ-casein initially, followed by covalent bonding. Rate of forming A_4 complexes increases with heating at 70°C.

During heating, Ca^{+2} binds to κ-casein and β-lactoglobulin, and may alter the structure and reactivity of these proteins (38). A_3 complexes formed by heating are less reactive with κ-casein when the solution contains Ca^{+2}. The β-lactoglobulin trimer forms more rapidly in the presence of Ca^{+2}.

Haque and Kinsella (39) investigated the change in hydrophobicity of κ-casein and β-lactoglobulin during heating. Using a fluorescent probe, 8-anilino-1-naphthalene sulphonic acid, they monitored a decrease in hydrophobicity with increased heating time. With a pH stat, exposure of acidic groups was monitored by titration with NaOH during heating. During heating, hydrophobic groups become less exposed while acidic groups are more exposed to the protein surface.

Heat Stability and Genetic Variants. Slight alterations in protein structure affect stability to heat. Evidence for this is differences in heat stability between genetic variants in milk proteins. Most genetic variants in milk differ by only one or two amino acids.

McLean et al. (58) studied the heat stability of milk proteins with respect to genetic variation and breed of cow. Concentration of β-casein and κ-casein correlate positively with heat stability in preheated concentrated skimmilk. Concentration of α_{s1}-casein and β-lactoglobulin correlate negatively with heat stability. The B variants of κ-casein and β-lactoglobulin are more heat stable than the A variants in concentrated skimmilk. Milk from Jersey cows is more stable to heat than milk from Friesians. Sawyer (73) found the C variant to be more heat labile than the other β-lactoglobulin variants. The A variant of β-lactoglobulin is more heat stable than the B variant. Following studies with NEM, Sawyer (73) calculated percent aggregation due to sulfhydryl/disulfide (SS/SH) reactions. Sixty-three percent of the aggregation in β-lactoglobulin A, 45% in β-lactoglobulin B, and 28% in β-lactoglobulin C is because of SH/SS reactions. Sawyer (73) believed difference in reactivity of SS and SH groups between variants is because of proximity to sites of amino acid substitution between variants. Gough and Jenness (32) found β-lactoglobulin A more stable than the B variant when heated at 74°C for 30 min, in agreement with Sawyer (73). This was observed with purified β-lactoglobulin in buffer and also in milk. When studying functional properties of whey proteins, variation in protein genetic variants of the milk supply could alter protein reactivity and functionality.

Heat Stability of Skimmilk Retentate. Sweetsur and Muir (83) found skimmilk UF retentate more heat stable than concentrate prepared by evaporation. Unlike evaporated skimmilk, heat stability of UF retentate is not improved by forewarming at 90°C for 10 min. Greater heat stability after removal of smaller molecules like lactose and ions during UF suggests that these constituents may influence heat coagulation of protein. Because skimmilk retentate has more protein, less lactose, and better heat stability than evaporated skimmilk, it has potential for use as a sterilized milk product.

Functional Properties of Whey Proteins Affected By Heat

Whey protein concentrates (WPC) are used for their functional properties in many foods. Because whey proteins are sensitive to heat induced reactions, concentrates with a variety of functional properties result. It is possible to customize WPC's for nearly any application. Gelation, emulsification, whipping, and foaming properties may all be exploited by the users of WPC's.

Gelation. Hillier et al. (46) found that solutions of 10% whey powder form stable gels when heated at 80°C. The whey powder they used contained 53.4 to 86.8% protein, and solutions were adjusted to pH 8.0 before heating. They concluded that gel structures form by intermolecular disulfide bonds. Gels dissolve when known disulfide interrupters, dithiothreitol or mercaptoethanol are added. Urea addition does not disrupt the gels. Gelling time is lowest below pH 6.0, although the gel is described as a coagulum. Since sulfhydryl (SH) groups are apparently involved in gel formation, it is likely that gelling time is related to -SH content of the whey powders. Two different whey powders were tested, using NEM and PCMB to block disulfide formation. With increasing amounts of blocker, gelling time increases. As available sulfhydryl groups decrease, disulfide formation is not possible, and gelling time increases.

Haggett (36) formed gels by heating solutions containing 10% whey protein. At pH 6.0, gels form when the heating temperature reaches 78 to 81.5°C. Gelling time decreases with increasing temperature between 60 and 90°C (45).

Disulfide bonds are formed by oxidation of sulfhydryls to disulfides, an oxidation-reduction reaction. Presence of oxygen could enhance the reaction. To evaluate the role of oxygen in disulfide formation, whey powder solutions were heated under nitrogen gas. Contrary to what was expected, gelation time decreases in the absence of air (20).

Although Hillier et al. (46) showed a relationship between gelling rate and sulfhydral content, other factors also affect gelation. When gelling time for nine different whey powders was plotted vs. total sulfhydryl content, data points were widely scattered.

Other unmeasured components of the whey powders could also contribute to gelation. Whey powders used in this study varied from .18 to 24.40% lactose and 2.43 to 10.5% ash. Calcium (in ash) and lactose affect thermal denaturation of whey proteins (19) and could affect gelling time. Gelation time of whey protein solutions varies with pH and temperature (45). Gelation occurs most rapidly between pH 5.0 and 5.2.

Gelation of whey proteins is usually compared with egg white protein. To obtain a gel as firm as egg white, 1% WPC was heated with .12% sodium hexametaphosphate at 55.5°C for 2 min. (62). Typically, egg albumen provides a gel structure when cooked. One advantage of using egg albumen is that it gels at 60°C whereas whey protein may need to be heated to 80°C before gelling occurs (59). When baking cakes, if gelation occurs at a higher temperature, the foam may collapse before gels form to stabilize it.

When WPC prepared by ultrafiltration without diafiltration (UWPC) was adjusted to pH 8.5, gelation temperature was 69-69.5°C (36). This is only 1.0-1.5°C higher than that of egg white. Gel strength at pH 6.0 is less than egg white but at pH 8.5, gel strength for WPC is greater than egg white.

Heating skim milk with 1.5% whey protein at 85°C for 5 min results in a custard-like gel, firm enough to stand alone without leakage (57). A gel of similar firmness formed with twice the amount of egg albumen.

Protein Gelation in Process Cheese. In the U.S. process cheese market, it is desirable to have a product that melts well when placed on a hamburger. Process cheese is used extensively in the fast food industry and strict specifications for melting quality must be achieved. In Europe and Australia, meltability is of little concern in the process cheese industry. Some manufacturers desire a product of limited meltability. A patent was issued for making melt-resistant process cheese by adding 1-20% of a protein that coagulates when heated to processing temperatures of 70-80°C (77). As an excellent addition to process cheese, whey protein could provide desired melt-resistance.

For certain food applications, process cheese melts and runs out of the food. Melt-resistant process cheese would be well suited for cheese-containing hot dogs. Currently, the cheese flows out of the hot dogs while cooking and ends up in the frying pan or boiling water. Schulz (77) suggested using melt-resistant cheese for dishes such as bread with cheese (Welsh Rarebits), scrambled eggs, or meat and fish dishes with sliced cheese fillings.

Where meltability is a desired characteristic in process cheese, it is a disadvantage to use Cheddar cheese for manufacturing made from ultrafiltered milk (UF curd) as described by Ernstrom et al. (29). Decreased meltability in process cheese made from UF curd is caused by the increased concentration of whey proteins compared to traditional Cheddar cheese. Savello (72) showed that added whey protein in a model process cheese causes decreased meltability. Whey proteins probably gel during heating of process cheese, resulting in melt-resistant cheese as suggested by Schulz (77).

Emulsification. Whey protein concentrates (WPC) have potential as emulsifying agents for fats and oils. WPC is a better emulsifier than nonfat dry milk but inferior to casein (59). WPC emulsifies 880 g oil per g WPC compared to 470 g oil for nonfat dry milk and 1,364 g oil per g casein.

Foaming and Whipping. WPC's have been studied for their suitability in producing foams and whips. Nakai and Li-Chan (62) studied whippability and foam stability in WPC. A comparison was made of the protein structure of whey proteins compared to egg white. Whey proteins have enough SS bonds, adequate hydrophobicity, but fewer SH bonds than egg white protein. Reduction of a few SS bonds in whey proteins could make the functional properties more like egg white protein. WPC was modified by treatment with cysteine, alcohol, linoleate, proteinases, polyphosphate, activated carbon, and thiolation. Treatment with cysteine and pepsin produces the highest overrun score. The overrun of 183% for WPC exceeds the 176% overrun obtained with egg white. Foam stability is similar to that of egg white protein.

Whippability of WPC can be improved by heat treatment and pH adjustment (57). Heat treatment enhances whippability by partially denaturing the whey protein, resulting in increased water holding capacity. Cottage cheese whey, heat treated at 71.1°C for 15 min and adjusted to pH 6.0, forms a white, fine grained foam with 300% overrun and stability of 6-7 h. Cheddar cheese WPC also forms stable whips if fat content is less than 1%. Concentrates with 2-3% fat form foams with less overrun and stability than WPC's with less than 1% fat.

Although heat treatment has been used to improve whippability of WPC (57), heat treatments above 70°C can cause a decrease in solubility, foamability, and emulsification (21). Haggett (36) found that cheese WPC had better whippability if heated at 50-60°C for 5-10 min. Heating above 65°C impairs whipping ability. Whipping properties of casein WPC are not improved by heating at 50-60°C.

Calcium concentration affects whip overrun and stability (22, 48). When calcium ions are replaced by sodium ions in WPC's, overrun and stability decrease with increased sodium replacement (48). Either calcium adds stability to protein structure, enhancing foam formation or sodium affects protein negatively, altering foam formation.

Haggett (36) compared whipping properties of whey protein concentrate obtained from casein manufacture prepared by ultrafiltration (UWPC) or ultrafiltration with diafiltration (DWPC). Whips formed from UWPC solutions containing 10% protein have overruns that exceeded that of egg white. Stability is less for WPC's than for egg white except when pasteurized whey is used and the pH adjusted to 8.5. Sucrose addition inhibits overrun formation for all WPC's and for egg white. Whips made from DWPC have lower overrun and less stability than those made from UWPC. Foaming power is determined by injecting 200 ml air into 100 ml of a 1% protein solution of WPC over 2 min (36). Volume of air trapped in the foam was measured by comparing the volume of foam and remaining liquid. A ratio of trapped air to injected air was used to

express foaming power. Foaming power and stability are much greater for WPC's made by ultrafiltration alone (UWPC), than when diafiltration is used (DWPC).

According to Haggett (36), heat treated cheese WPC's all have poor foaming properties. Poor foaming ability was attributed to the butterfat in Cheddar cheese whey compared to casein whey which is practically fat free.

Acidification: Effect of Calcium

Addition of disodium oxalate to rennet casein model process cheese made with disodium phosphate (DSP) or tetrasodium pyrophosphate (TSPP) results in improved meltability (72). Model process cheese made with rennet casein melts better than acid casein model cheese when citrate is used as emulsifying salt. Since rennet casein contains higher levels of calcium than acid casein, and citrate and oxalate are known calcium binders, poor meltability might be associated with high calcium levels.

Ernstrom and Anis (28) showed improved meltability in process cheese made from ultrafiltered milk, after preacidification to pH 6.4, 6.2, 6.0, and 5.8. They believed the increase in meltability with a decrease in pH is because of an increasing amount of calcium removed. To test the hypothesis, retentate made from acidified milk was fortified with calcium to increase the concentration from .4 to .6% (2). Meltability decreases with calcium fortification. In calcium fortified process cheese, meltability is improved by increasing sodium citrate from 2.5 to 5.0%. It was concluded that removal of calcium by preacidification of milk or chelation with citrate improves meltability of UF process cheese food.

Keller et. al. (49) found that meltability decreases with increasing pH of mozzarella cheese made by direct acidification. Cheese made from milk acidified with citric acid has better meltability than when acidified with malic, acetic, hydrochloric or phosphoric acid. Acidification of milk with citric acid produces cheese with less calcium than when acidified with other acids. However, curd moisture was higher when citrate or malic acid

was used. They concluded that this was because of the influence of the anionic species of acid on solvation of cheese protein.

Citrate used as an emulsifying salt in process cheese may increase bound water associated with casein. Nakajima et al. (63) found that citrate increases bound water with both calcium caseinate containing colloidal phosphate and colloidal-phosphate-free casein. In determining melting quality of process cheese food, protein solvation may be more important than chelation of calcium.

Ultrafiltration, Process Cheese, and Whey Proteins

A principal advantage of ultrafiltration in cheese manufacture is recovery of the whey proteins in the retentate. When cheese curd is made as described by Ernstrom et al. (29), retentate is evaporated under vacuum until the product contains 38 to 39% moisture. All constituents of the retentate are recovered in the curd since syneresis does not occur. Although retention of whey protein increases yield of cheese curd, the curd functions differently when processed because of susceptibility of whey protein to heat denaturation. Minerals such as calcium and phosphorus contribute to protein stability and may affect rheological properties of process cheese made from UF curd.

METHODS AND MATERIALS

General Ultrafiltration Technique

Ultrafiltration was by a batch process using an Abcor spiral wound polysulfone membrane with a filtering surface area of 5 m^2. The nominal molecular weight cut-off was 10,000. Raw whole milk received a 16 s heat treatment prior to ultrafiltration. Milk was cooled to 4°C before acidification with concentrated HCl. After acidification, milk was equilibrated for 12-16 h before ultrafiltration. Milk was heated to 54°C and maintained at that temperature throughout the ultrafiltration process. Diafiltration involved adding deionized water to retentate at the same rate permeate was removed. Amount of diafiltration water was adjusted to control the buffer capacity/lactose ratio so the final pH of the fermented retentate was 5.1 ± .1. Following diafiltration, retentate was concentrated until 80% of the original milk weight was removed (5× concentrate). At all sampling points, rate of permeate flow was measured.

Milk Quality

One purpose of this study was to determine nutrient partitioning during UF of milk with high bacterial numbers. Three batches of milk with standard plate counts of <100,000; 500,000-1,000,000; and >2,000,000 were obtained. Following pasteurization at 72°C for 16 s, the pH was adjusted to 5.8, and milk was ultrafiltered to remove 60% of the original milk weight. It was diafiltered with deionized water equal to 55% of the original milk weight then reduced to 20% of the original weight. Samples of retentate and permeate were collected at the beginning, middle, and end of diafiltration and at the final 5× concentration. A sample of original milk was also saved. Retentates and permeates were analyzed for solids, fat, nitrogen, lactose, and non-protein nitrogen. Free fatty acids and rennet clottable nitrogen were determined in the milk and retentate. The raw milk was tested for pH, titratable acidity, somatic cell count, and standard plate count.

Varying Beginning Point of Diafiltration

The purpose of this experiment was to compare nutrient retention and recovery, water usage, and time expenditure while varying the beginning point of diafiltration. Milk was pasteurized at 72°C for 16 s and acidified to pH 5.8. Ultrafiltration proceeded to 60% volume reduction (VR), 65% VR, and 70% VR before beginning diafiltration. When diafiltration began at 60% VR, diafiltration water equaled 55% of the original milk weight. When concentrated to 65% VR before diafiltration, diafiltration water was 50% and for pre-concentration to 70% VR, rate of diafiltration was 38.5%. Samples were collected as was described in the milk quality study. Retentate, permeate, and milk samples were analyzed for solids, fat, lactose, calcium, ionic calcium, nitrogen, rennet clottable nitrogen, vitamin B_{12}, riboflavin, and phosphorus. Only 5× concentrate was tested for buffer capacity. The overall efficiency of the process was determined.

Heat Treatment and Process Cheese Food

The purpose of this experiment was to determine the effect of heat treatment and acidification prior to UF on nutrient recovery and retention. Effect of acidification and heating milk prior to UF, heating 5× concentrate, and temperature of cooking pasteurized process cheese food were evaluated with respect to meltability.

Study A: Acidification and Heat Treatment Prior to UF. Milk was heated to 60, 72, and 82°C for 16 s. Milk representing each heat treatment was divided into three batches, and adjusted to pH 5.8, 6.2, and 6.6 (unacidified). Diafiltration rates at 60% VR were 55% for pH 5.8, 44% for pH 6.2 and 36% for unacidified milk. Retentate was reduced to 5× concentration and fermented with .5% of a 2 strain *Streptococcus cremoris* culture (Miles Laboratories, Biolac Division) at 28°C for 16 h. Moisture was reduced to 36-38% in a swept surface vacuum evaporator at -76 kPa. Product temperature remained below 43°C. This product was referred to as UF cheese curd. Pasteurized process cheese food was made from UF cheese curd by a method similar to that of Ernstrom et. al. (29). Part

of the milk preheated to 72°C was made into Cheddar cheese by the traditional 4.25 h process (68). Pasteurized process cheese food was made by mixing UF cheese curd, sodium chloride, sodium citrate, and water in a 3 kg cheese cooker. After mixing, process cheese food was cooked to 82°C and immediately removed from the cooker. The final product contained 43-44% moisture, 2.5% sodium citrate, and 1.7% sodium chloride. Following cooling, retentate samples and process cheese were analyzed for solids, fat, protein, calcium, lactose, vitamin B_{12}, riboflavin, and phosphorous. Retentate was tested for ionic calcium and rennet clottable nitrogen. Cheese samples were analyzed for amino acids and meltability.

Study B: Effect of Preheating, Acidification, and Cooking Temperature. Study A was repeated with 70% diafiltration water for milk acidified to pH 5.8, 50% for milk at pH 6.2, and 38.5% for unacidified milk. Before UF, milk was preheated to 61, 72, or 83°C for 16 s. Each batch of process cheese food was cooked to 70, 76, or 81°C to determine the effect of cooking temperature on meltability.

Study C: Effect of Heating Retentate. Unacidified milk was heated to 72°C for 16 s, ultrafiltered to 60% VR, diafiltered 38.5% and concentrated to 5×. Retentate was divided into four equal portions. One batch was unheated and the others were heated to 61, 72, or 83°C for 16 s. Process cheese food was made as described in study B. Total solids, fat, protein, calcium, and phosphorus were measured in retentate and cheese. Process cheese food was tested for meltability.

Effect of Cooking Temperature on Meltability and Soluble Protein

Milk was pasteurized at 73°C for 28 s, heated to 54°C, and ultrafiltered until 70% of the original milk weight was removed (70% VR). Diafiltration was at constant volume, using deionized water equal to 30.25% of the original milk weight. Then, ultrafiltration continued until 20% of the original milk weight remained (5× concentration). Two batches of pasteurized process cheese food were prepared, one with samples taken when

the cooking temperature reached 66, 70, 74, 78, and 82°C. The other was sampled at 68, 72, 76, and 80°C. For replication, two more batches of cheese food were prepared and sampled at the same cooking temperatures. Meltability and soluble nitrogen at pH 4.6 were tested for all cooking temperatures.

Blends of UF Curd and Cheddar Cheese

Meltability of process cheese food made from UF curd can be improved by reducing the processing temperature. To meet the legal requirements, pasteurized process cheese food must be cooked at not less than 65.6°C (150°F) for not less than 30 s (14). Manufacturers of process cheese and process cheese foods use a higher cooking temperature primarily to inhibit spoilage. UF curd can be manufactured in a closed system, although Cheddar cheese for processing is likely to contain mold spores and undesirable bacteria. Higher cook temperature would be more important for processed Cheddar cheese than UF curd. If blended together, it would be desirable to give Cheddar cheese a separate, higher heat treatment before blending with UF curd.

Process cheese food was made by first heating Cheddar cheese to 82°C, cooling to less than 55°C, adding sodium citrate, water, and UF curd. Then it was cooked to 72°C, and immediately removed from the cooker to cool. In a second experiment, the same procedure was followed, except the final cook temperature was 68°C. Product composition was 42-44% water, 1.5% NaCl, and 2.5% sodium citrate. A blend of 27% aged and 73% mild Cheddar cheese was used.

Comparison of Olson and Price Melt Test with the Schreiber Method

After making process cheese food from blends of Cheddar cheese and UF curd, meltability was evaluated with both the Olson and Price (64) and Schreiber test (51). The Schreiber test was used to determine if the meltability would be acceptable by industrial standards.

Possible Mechanism for Decreased Melt by Disulfide Interaction

It was hypothesized that poor meltability of process cheese food made from UF curd with increased processing temperature was because of whey protein denaturation. Denaturation could be accompanied by molecular rearrangement and stabilized by formation of new disulfide bonds. To test this hypothesis, NEM was added to process cheese food before cooking began. Three levels of NEM were used, 5, 10, and 20×10^{-4} *M*. Additionally, process cheese food was made with sodium oxalate or NEM alone, and both chemicals together.

Chemical Analyses

Total Solids. Total solids in milk was measured using Association of Official Analytical Chemists Method number 16.032 (3). A modification of the method was necessary to ensure adequate drying of 5× retentates. After weighing, concentrated retentates were diluted with distilled water to distribute sample evenly over the bottom of the aluminum pan. Total solids in cheese was determined by the method of Price et al. (69).

Fat. Fat was analyzed by the Mojonnier modification of the Roese-Gottleib method (81).

Nitrogen Analysis. Nitrogen analysis was by semi-micro Kjeldahl using a selenium catalyst, concentrated sulfuric acid, and a Tecator automatic distillation and titration apparatus. Protein was estimated using percent nitrogen × 6.38 (3).

Nonprotein Nitrogen. Nonprotein nitrogen (NPN) was determined by a modification of the method of Cerbulis and Farrell (12). An equal volume of milk or permeate and 24% trichloroacetic acid were mixed together. Concentrated milk and retentate were first diluted with water to the same protein and fat content as the original

milk. The supernatant was filtered with Whatman #4 filter paper. Protein analysis was performed on the supernatant as described above.

Rennet Clottable Nitrogen. Rennet clottable nitrogen required first diluting retentate with H_2O to the concentration of milk (5× retentate diluted 1:5). Calcium chloride (400 ml of .1 *M*) and 7.3 rennin units of chymosin (The New Zealand Co-op. Rennet Co. Ltd.) were added to 5 ml milk or diluted retentate at 32°C. Coagulation occurred in 10-20 min, followed by cutting the curd and centrifugation at 1,400 × g for 15 min. Whey was removed and tested for nitrogen. Rennet clottable nitrogen was calculated by subtracting whey nitrogen from total nitrogen.

Soluble Nitrogen at pH 4.6. Soluble nitrogen was determined by a modification of the method of Vakaleris and Price (86). Cheese food was finely grated and 5 g sample accurately weighed into a small plastic bag. Cheese was blended with 20 ml .5 *M* sodium citrate for 10 min. The slurry was incubated for 60 min in a 40°C water bath. Samples were transferred by washing with water into 100 ml volumetric flasks. The pH was adjusted to 4.6 by adding 12.0 ml of 1 N HCl. Flasks were brought to 100 ml by adding water. After mixing, some of the fluid was centrifuged in a clinical centrifuge at 5,000 × g. The supernatant was filtered with Whatman #42 filter paper. Kjeldahl nitrogen was determined on the filtered supernatant fluid and on cheese food samples. Soluble protein at pH 4.6 was expressed as a percent of total protein.

Lactose. Lactose determination was by the method of Shaffer and Somogyi (78).

Free Fatty Acids. Free fatty acids were determined by measuring acid degree value using the method of Driessen et al. (24) as summarized by Koops and Klomp (50).

Mineral Analysis. For mineral analysis, approximately 1 g samples were weighed into 100 ml straight tubes. After adding 10 ml nitric acid, samples were ashed at 110-120°C for 48 h. Nitric acid digests were diluted to 50 ml with deionized distilled water. Total calcium was determined by atomic absorption spectroscopy after diluting all samples in 1% lanthanum from La_2O_3 or $LaCl_3$. Lanthanum was used to prevent

interference with phosphorus during atomic absorption spectroscopy. This method was recommended by Van Loon, although he used .14% lanthanum (88). Phosphorus was measured by the phosphomolybdate method (1). Ionic calcium was determined with an ion specific electrode (17). For ionic calcium, samples were first centrifuged, milk at 47,000 × g for 80 min and 5× retentate at 109,000 × g for 60 min, to remove fat and casein. A sample of 1-2 ml of clear milk serum was diluted to 25 ml and .5 ml 4 *M* KCl was added to adjust ionic strength. Readings were taken after 2 min. equilibration time. Permeate was analyzed directly after adding 4 *M* KCl.

Riboflavin. Riboflavin was measured using an extraction procedure similar to that of Lumley and Wiggins (54). Ten milliliters of milk or retentate was mixed with 10 ml .1 N HCl and autoclaved at 109-116°C for 30 min. After adding 5 ml 1.6 *M* acetate buffer (217.6 g sodium acetate trihydrate and 60 ml glacial acetic acid diluted to 1 L with distilled water) samples were filtered through Whatman #5 filter paper. Before high performance liquid chromatography (HPLC) injection, samples were filtered again with filter paper of.45 μm porosity. After extraction, riboflavin was quantitated by HPLC using the method of Ashoor et al. (4). Column dimensions were 35 × 7 mm with octadecasilane packing of 3 μm particle size, purchased from Perkin Elmer. Fluorescence detection was with excitation at 390 nm and emission at 520 nm. Standards were obtained from Sigma Chemical Co.

Vitamin B_{12}. Vitamin B_{12} analysis was performed by a competitive binding assay using a commercially prepared kit (13). Kits were obtained from Diagnostic Products, Los Angeles, CA, and Bio-Rad Labs, Richmond, CA.

Amino Acid Analysis. Amino acid analysis was by HPLC using the method of Bidlingmeyer et al. (8). Milk or retentate was hydrolyzed in 6 N HCl for 20 h at 110°C. Protein concentration in the hydrolysate was 2 mg/ml, except for cheese when 5 mg/ml was used. Following hydrolysis, all milk and retentate samples were cleaned-up by solid

phase extraction, using a Sep-Pak cartridge (Waters Associates). Amino acids were derivatized with phenylisothiocyanate (PITC) and detected at 254 nm.

Polyacrylamide Gel Electrophoresis. PAGE was done in a vertical slab gel system using the method of Hames (37) as described by Yiadom-Farkye (91) with modifications for separation of whey proteins. The resolving gel contained 9% cyanogum 41 (acrylamide and N,N'-methylene-bis-acrylamide, Sigma Chemical Co.) and stacking gel 5% cyanogum 41. Urea was not used in the gels and neither urea nor mercaptoethanol were added to the samples. Samples were prepared by weighing .2 g finely grated cheese into a small test tube. After adding 1 ml stacking gel buffer, tubes were vortexed and incubated for 40 min at 40°C. Then, .1 ml of 1 *M* acetic acid was added to reduce the pH to 4.6. Tubes were centrifuged at 5,000 × g for 15 min. The supernatant was filtered through a .45 µm filter and 50 µl tracking dye was added to .5 ml sample. Gels were run at constant current using 20 mA per 1.5 mm thick gel.

Meltability Tests

Olson and Price Melt Test. Meltability tests were a modification of the method of Olson and Price (64) as described by Savello (72). A 15 ± .1 g cylinder of cheese was placed in one end of a glass tube measuring 32 mm in diameter by 25 cm long. The end containing cheese was closed with a rubber stopper. Cheese was tempered at a 45 degree angle in an incubator at 30°C for 2 h. Then, tubes were placed horizontally in a 110°C oven for 50 min. Melting distance was the difference between the initial upper edge of the cheese plug and leading edge of the melted cheese.

Schreiber Melt Test. Meltability was tested as described by Kosikowski (51) and scored with a grid of concentric circles (65) on a scale of 1 to 10. A score of 1 was no melt, and 10, the maximum possible. Disks of cheese 4.8 mm thick by 41 mm diameter were placed in a glass petri dish and heated for 5 min at 232°C. Melt was scored following cooling at room temperature for 30 min.

Statistical Analysis

Data for all studies were analyzed by analysis of variance using software from Statistical Analysis System (Cary, N.C). Analysis of variance and regression tables are included in the appendix. Mean comparisons were made following a significant F test, using Fishers LSD (60). Data on amino acids in retentate and recovery of nutrients following preheating and acidification were from replicated experiments, so calculated Mean Square Error (MSE) should represent experimental error. An α level of .05 was used to determine significance. All other data were from experiments that were not replicated, and experimental error was approximated by using error associated with subsampling. Subsampling probably showed less error than true replication, so the MSE was probably underestimated and F tests should be interpreted with that consideration. To compensate for an underestimated MSE, an α level of .01 was used to determine significance.

RESULTS AND DISCUSSION

Amino Acid Analysis

Amino acid analysis of milk and retentate samples taken during ultrafiltration is presented in Table 1. Each mean value in the table was obtained from duplicate samples of three ultrafiltration runs. Analysis of variance was used to determine if amino acid composition of milk changed during ultrafiltration. During ultrafiltration, only methionine and lysine had significant differences as g/100 g protein. The sample taken at 40% VR had the most lysine. Methionine was highest at 20 and 40% VR. This may have been a measurement problem since methionine is easily oxidized and lysine would be involved in Maillard browning reactions with lactose. For all amino acids, including lysine and methionine, there were no differences in protein between milk and 5× retentates with respect to grams of amino acids per 100 g protein. Ultrafiltration of milk did not cause a change in amino acid composition of milk protein. Hence, protein quality is not changed by ultrafiltration to a 5× concentrate.

Amino acid composition of three pasteurized process cheese food samples made from UF retentate and one Cheddar cheese is presented in Table 2. All were made from the same milk. From statistical analysis, aspartic acid and lysine were significantly different. Lysine was different for all cheeses, lowest in natural Cheddar cheese and highest in UF pasteurized process cheese food made from unacidified milk. Cheddar cheese was lower in aspartic acid than UF cheese made from acidified milk but not significantly different from cheese made from unacidified milk. We found no differences for fourteen of sixteen amino acids. Cheese made from UF retentate has an amino acid composition similar to Cheddar cheese. Of the two amino acids that were significantly different, lysine was the only essential one.

Table 1. Amino acid analysis of milk concentrated by ultrafiltration to a 5× concentration without diafiltration.

	Percent of Milk Removed (% VR)				
Amino Acid	0	20	40	60	80
	(g/100 g protein)				
Asp	7.41	6.34	6.58	6.33	6.68
Glu	21.75	21.82	20.40	22.34	22.51
Ser	6.85	5.89	6.15	5.35	5.82
Gly	1.90	1.70	1.79	1.82	1.80
His	2.93	2.72	2.73	3.10	2.91
Arg	4.38	4.13	4.02	4.79	4.42
Thr	4.72	4.29	4.28	5.02	4.90
Ala	3.47	3.17	3.21	3.29	3.39
Pro	10.53	10.56	10.09	10.45	10.45
Tyr	6.74	6.90	6.64	7.18	7.32
Val	5.51	5.76	5.90	5.82	5.60
Met	2.32^{bc}	2.89^{a}	2.64^{ab}	2.39^{bc}	2.27^{bc}
Ile	3.87	4.15	4.09	4.05	4.24
Leu	8.47	8.77	8.57	8.34	7.91
Phe	2.82	3.07	3.39	2.92	2.85
Lys	5.46^{b}	6.95^{b}	8.61^{a}	5.92^{b}	6.03^{b}

^{abc}For a particular amino acid, means with the same letter are not significantly different at α=.05 using Fishers protected LSD.

Table 2. Amino acids in Cheddar cheese and UF pasteurized process cheese food

		UF Pasteurized Process Cheese Food		
	Cheddar	pH 5.8	pH 6.2	pH 6.6
Amino Acid	Mean(SEM)[e]			
Asp	6.86(.10)[a]	7.56(.09)[b]	7.55(.02)[b]	7.34(.09)[ab]
Glu	21.29(.57)	20.85(.12)	21.57(.05)	20.52(.13)
Ser	5.74(.05)	5.93(.10)	5.55(.03)	5.85(.04)
Gly	1.64(.0005)	1.63(.03)	1.61(.01)	1.61(.04)
His	2.89(.04)	2.53(.03)	2.61(.003)	2.54(.12)
Arg	4.61(.06)	4.29(.06)	4.28(.06)	3.87(.16)
Thr	3.80(.01)	4.34(.12)	4.35(.13)	4.08(.08)
Ala	2.94(.11)	2.99(.07)	3.13(.09)	3.13(.06)
Pro	9.84(.005)	9.24(.22)	9.80(.03)	9.76(.43)
Tyr	5.94(.15)	5.29(.02)	5.42(.11)	5.28(.07)
Val	5.52(.04)	5.51(.12)	5.59(.05)	5.67(.28)
Met	3.34(.01)	2.85(.16)	2.85(.41)	2.57(.18)
Ile	4.32(.04)	4.41(.03)	4.46(.05)	4.64(.12)
Leu	9.43(.24)	9.41(.01)	9.31(.04)	9.32(.002)
Phe	5.16(.34)	5.03(.04)	4.48(.09)	4.79(.04)
Lys	6.62(.07)[a]	7.94(.06)[b]	7.25(.06)[c]	8.74(.16)[d]

[abcd] For a particular amino acid, means with the same letter are not significantly different at α=.01 using Fisher's protected LSD.

[e]Amino acid concentration is in g amino acid/100 g protein. SEM is standard error of the mean.

Milk Quality Study

Nutrient values of 5× retentates prepared from milk with varying bacteriological qualities were compared by analysis of variance (Table 3). Retentate from milk with the highest bacterial count, 7.8×10^6 CFU/ml, had the lowest protein and total solids content. In Table 4, initial solids and protein content of the three milk samples are shown. Milk with 7.8×10^6 CFU/ml contained less solids than the other two lots initially and after concentration to 5× (Figure 1a). Total solids in permeate was nearly identical for all treatments (Figure 1b). It would be logical for final solids concentration in the retentate to also be less since the initial value was lower. Initial protein concentration was lowest in milk with 8.4×10^5 CFU/ml, although final protein concentration in the retentate was greatest for that treatment. (Tables 3, 4, Figure 2). This batch of milk was probably concentrated further than milk with 7.8×10^6 or 4.4×10^3 CFU/ml.

Table 3. Nutrient concentration and recovery in 5× retentate after UF of milk with high bacterial counts.

	Bacterial Colony Counts (CFU/ml)					
	7.8×10^6		8.4×10^5		4.4×10^3	
Component	Conc	Recov(%)	Conc	Recov(%)	Conc	Recov(%)
Protein(%)	13.39[a]	94	15.83[b]	102	14.66[c]	89.4
Lactose(%)	1.99[a]	9.2	2.19[b]	7.5	1.53[c]	6.2
NPN(mg/100g)	12.89[a]	10.1	10.38[b]	6.9	9.80[b]	6.1
Solids(%)	31.83[a]	56.9	40.15[b]	55.2	37.5[c]	55.7
FFA(ml 1 N KOH/100 g fat)	2.37[a]	36.3	.87[b]	18	.84[b]	15.9

[abc]For a particular component, means with the same letter are not significantly different at α=.01 using Fishers protected LSD.

Table 4. Nutrient composition, somatic cell count, pH, and titratable acidity for milk with high bacterial loads.

Component	Bacterial Colony Counts (CFU/ml)		
	7.8×10^6	8.4×10^5	4.4×10^3
Solids(%)	11.56	11.93	12.44
Lactose (%)	4.46	4.95	4.53
Protein (%)	2.943	2.814	3.01
NPN (mg/100 g)	26.35	24.68	29.39
pH	6.64	6.58	6.62
Somatic cells/ml	1.81×10^6	5.28×10^5	2.08×10^5
T.A.(%)	.136	.141	.155
FFA (ml 1 N KOH/100 g fat)	1.833	.798	.968

Because of difficulty in concentrating different batches of milk to the same factor such as 5×, it is useful to report nutrients as percent recovery. Recovery was calculated using the following equation:

$$\text{Percent Recovery} = \frac{\text{CF(nutrient)}}{\text{CF(fat)}} \times 100$$

CF(nutrient) or CF(fat), concentration factor, is the concentration in the final retentate divided by the concentration in the original milk. Recovery of nutrients was based on the assumption that fat was 100% retained by the membrane. Results of analysis of permeate in our laboratory showed no detectable fat passing the membrane, which was also determined by Yan et al. (90). Recovery, as defined here, is similar to yield as determined by Glover (35) and retention coefficient as described by Green et al. (34). The CF(fat) for milk with 8.4×10^5 CFU/ml was 6.1, compared to 4.84 for 7.8×10^6 CFU/ml and 5.45 for 4.4×10^3 CFU/ml. Since CF(fat) for milk with 8.4×10^5 CFU/ml was 6.1, final protein and solids concentration in retentate were greater than in the treatments concentrated to 4.84 or 5.45 ×. Protein concentration in the permeate was lower for the milk with 8.4×10^5 CFU/ml (Figure 2b), so greater concentration in the retentate could also be because of less passage through the membrane.

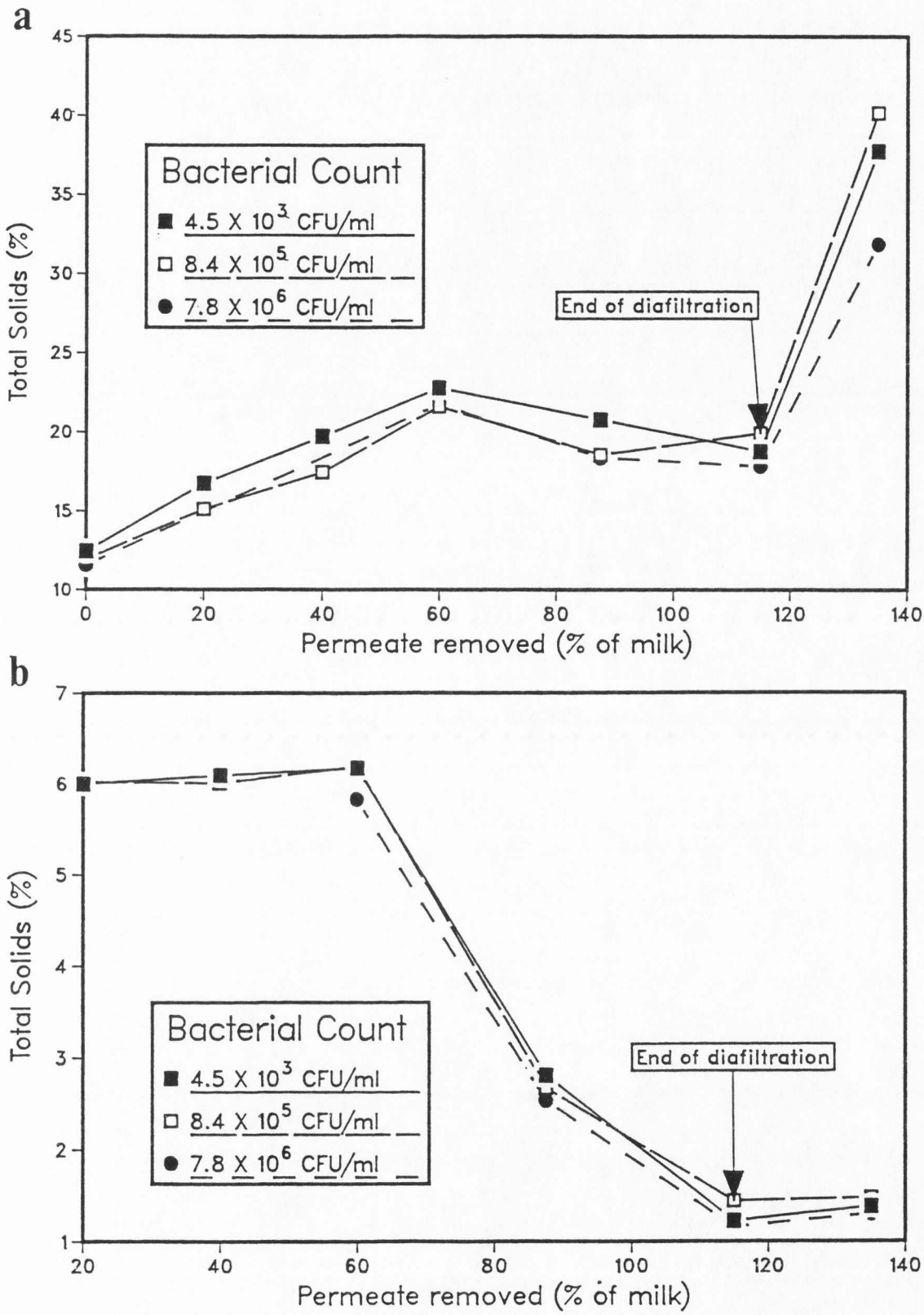


Figure 1. Total solids in (a) retentate and (b) permeate during UF of milk with high initial bacterial loads.

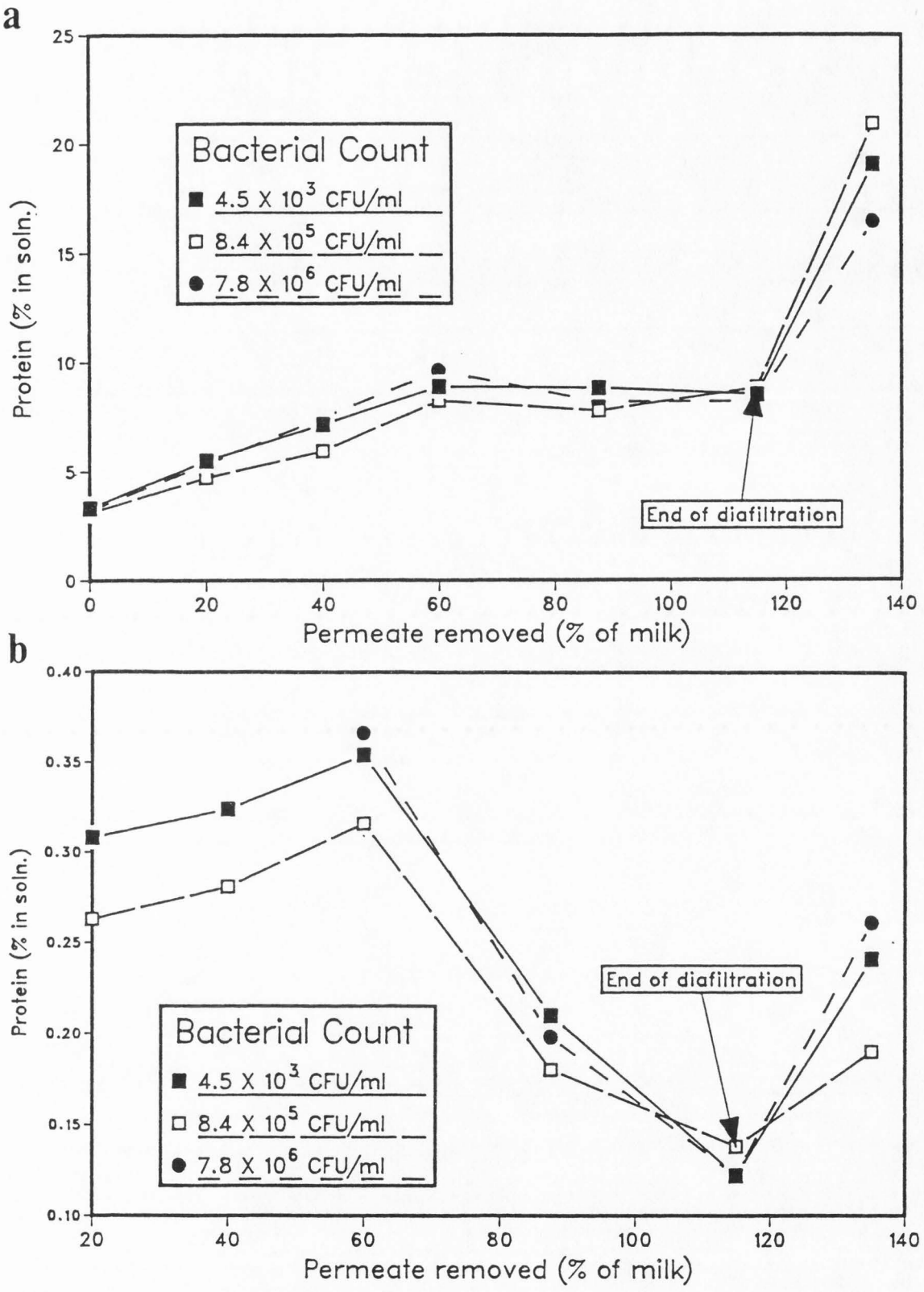


Figure 2. Protein in (a) retentate and (b) permeate during UF of milk with high initial bacterial loads.

Glover (35) stated that retention coefficients for molecules partially retained like lactose, should be expressed on the basis of the water phase. We have done this by expressing concentration of nutrients as percent in solution.

$$\% \text{ in Solution} = \frac{\% \text{ nutrient}}{\% \text{ moisture} + \% \text{ nutrient}} \times 100$$

Retention factor, R, as defined by Glover (35) is the ability of a membrane to retain a compound. Retention coefficients are expressed as percent retention.

$$\% \text{ Retention} = \frac{Cf\text{-}Cp}{Cf} \times 100 = 1 - \frac{Cp}{Cf} \times 100$$

Cf = concentration (%) of molecule in retentate.
Cp = concentration (%) in permeate.

Retention factor was defined identically by Yan et al. (90) but termed "rejection coefficient".

Table 3 includes information on percent recovery of nutrients. Percent recovery of solids was slightly higher in retentate made from UF of milk with 7.8×10^6 CFU/ml. Protein recovery was lowest in milk with the lowest bacterial numbers. It was not possible to tell if these differences were because of bacteriological quality, since the three lots of milk had numerous other differences (Table 4). With respect to protein and solids, there were no differences in final concentration or recovery in retentate attributable to bacterial count.

Initial free fatty acid (FFA) content in milk (Table 4), FFA recovery, and concentration in retentate were all greatest for the milk with 7.8×10^6 CFU/ml (Figure 3, Table 3). This milk and permeate removed during UF had an odor characteristic of free fatty acids. High FFA content was probably because of lipolysis occurring during improper cooling of the milk. FFA content in milk (Table 4), recovery, and concentration in retentate (Table 3) were practically equivalent for milk of $<10^6$ CFU/ml.

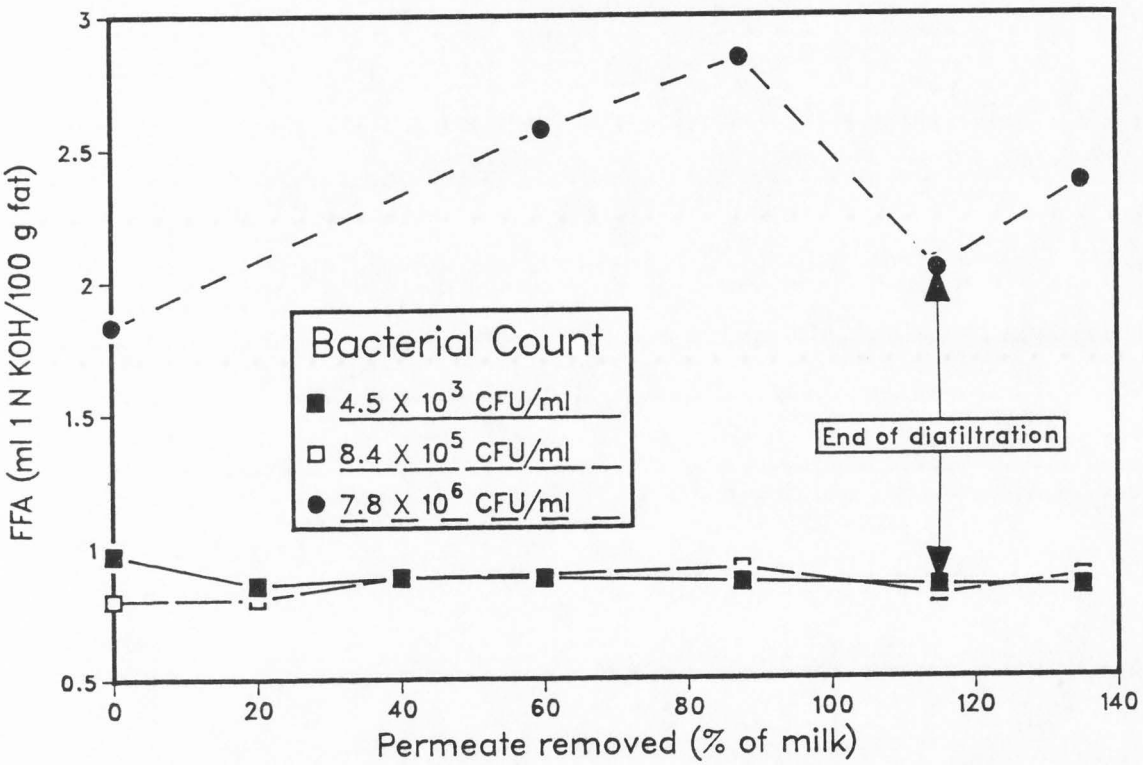


Figure 3. Free fatty acid content in milk with high initial bacterial load compared with the control (4.5×10^3 CFU/ml).

Initial pH, somatic cell count, and titratable acidity for the three batches of milk is in Table 4. Milk with high bacterial counts had higher somatic cell counts. This is typical for milk from cows with mastitis (89). Titratable acidity was highest in the best quality milk, probably because the milk had a higher solids and protein content. The acidity and buffer capacity of milk is determined mainly by proteins, phosphates, and citrates (89). Milk pH was within or near the normal range of 6.6-6.8 (89).

Nonprotein nitrogen (NPN) as percent of total nitrogen is shown for retentate and permeate in Figure 4. In both retentate and permeate, NPN as percent of total nitrogen decreased during UF. Percent of total nitrogen as NPN decreased in retentate because small peptides and amino acids passed through the membrane during UF, concentrating the protein and removing NPN. In permeate, percent of total nitrogen as NPN decreased because of dilution with diafiltration water. Final concentration of protein as percent of total nitrogen increased in both retentate and permeate. Green et al. (34) and Glover (35) also reported that protein constituted a higher proportion of total nitrogen as concentration progressed.

Retention of NPN increased as milk was concentrated (Figure 5) even though concentration of NPN as percent total nitrogen decreased (Figure 4a). Lactose concentration during UF and percent retention are included in Figures 6 and 7. These results are similar to those reported by Bastian (7). Before diafiltration, lactose concentration in both permeate and retentate was constant, then decreased during diafiltration and increased slightly after diafiltration was ended. Percent retention of lactose increased during diafiltration. As retentate was diluted with water, a greater difference in concentration between retentate and permeate was seen. Final lactose concentration in retentate was highest from the milk with 8.4×10^5 CFU/ml (Table 3). This could be because of a greater initial lactose concentration (Table 4). Milk with the lowest bacterial count had the lowest final lactose concentration and percent recovery. Milk with the highest count had the greatest lactose recovery in retentate but was between

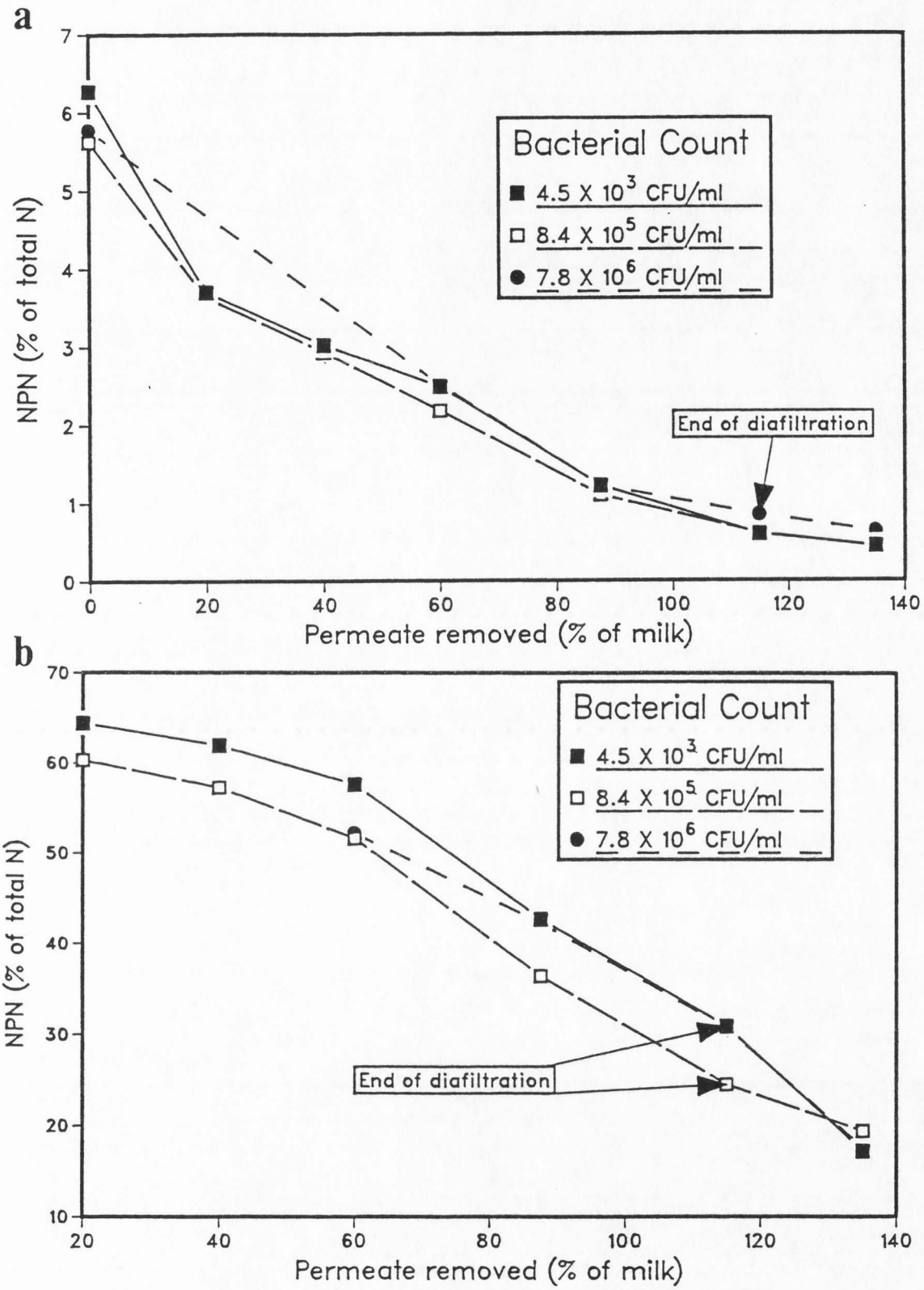


Figure 4. NPN as a percentage of total nitrogen in (a) retentate and (b) permeate during UF of milk with high initial bacterial loads.

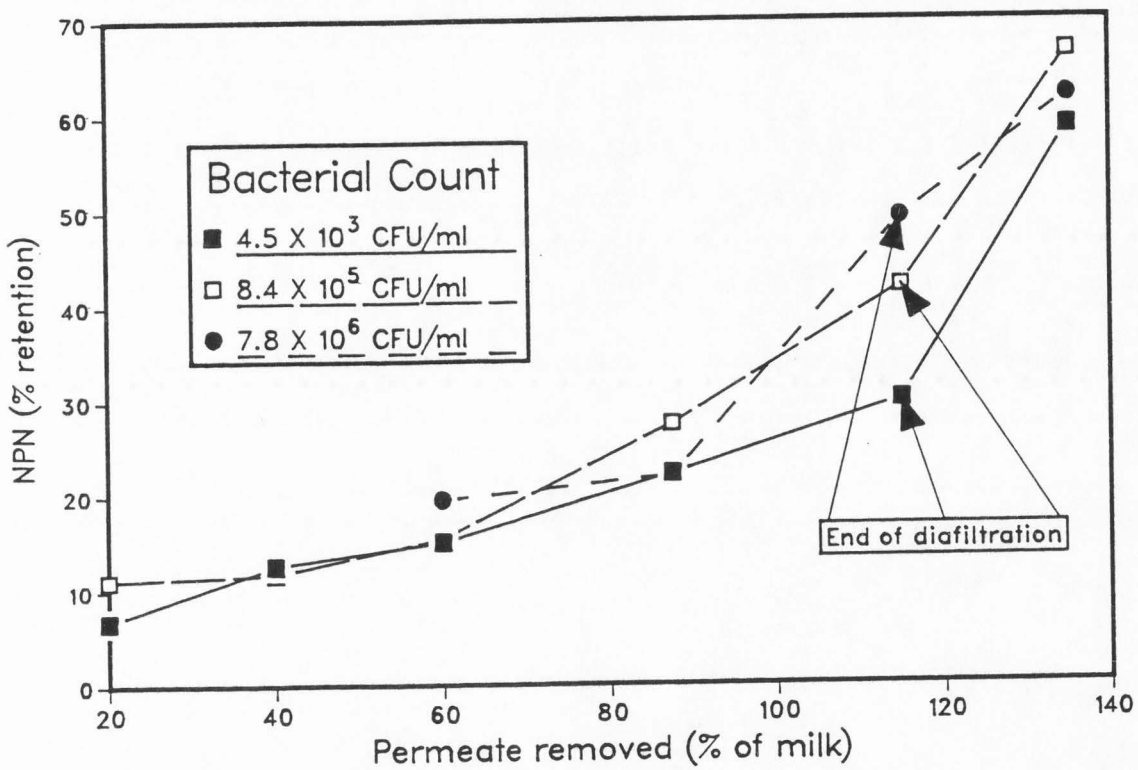


Figure 5. Retention of NPN during UF of milk with high initial bacterial loads.

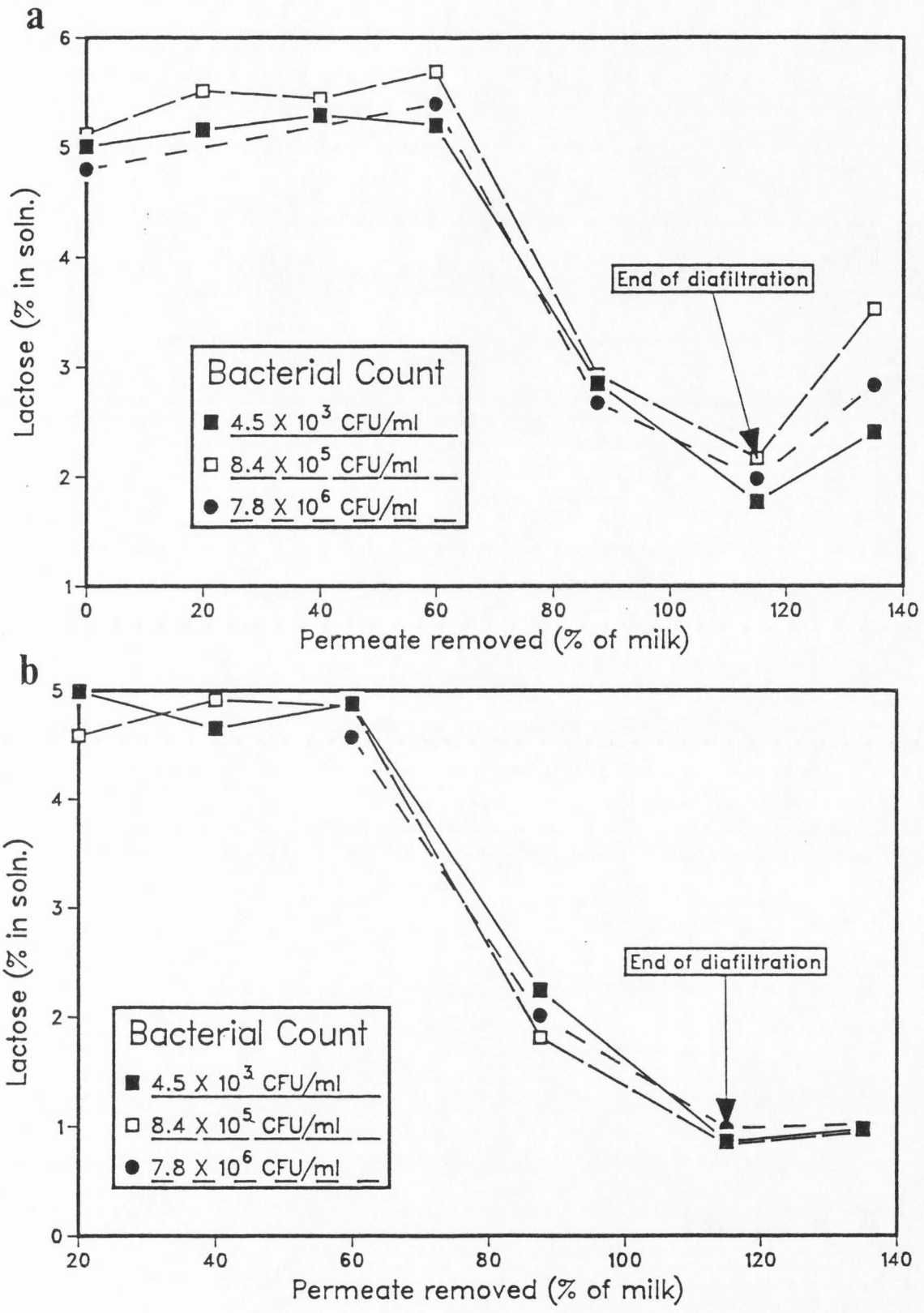


Figure 6. Lactose concentration in (a) retentate and (b) permeate during UF of milk with high initial bacterial loads.

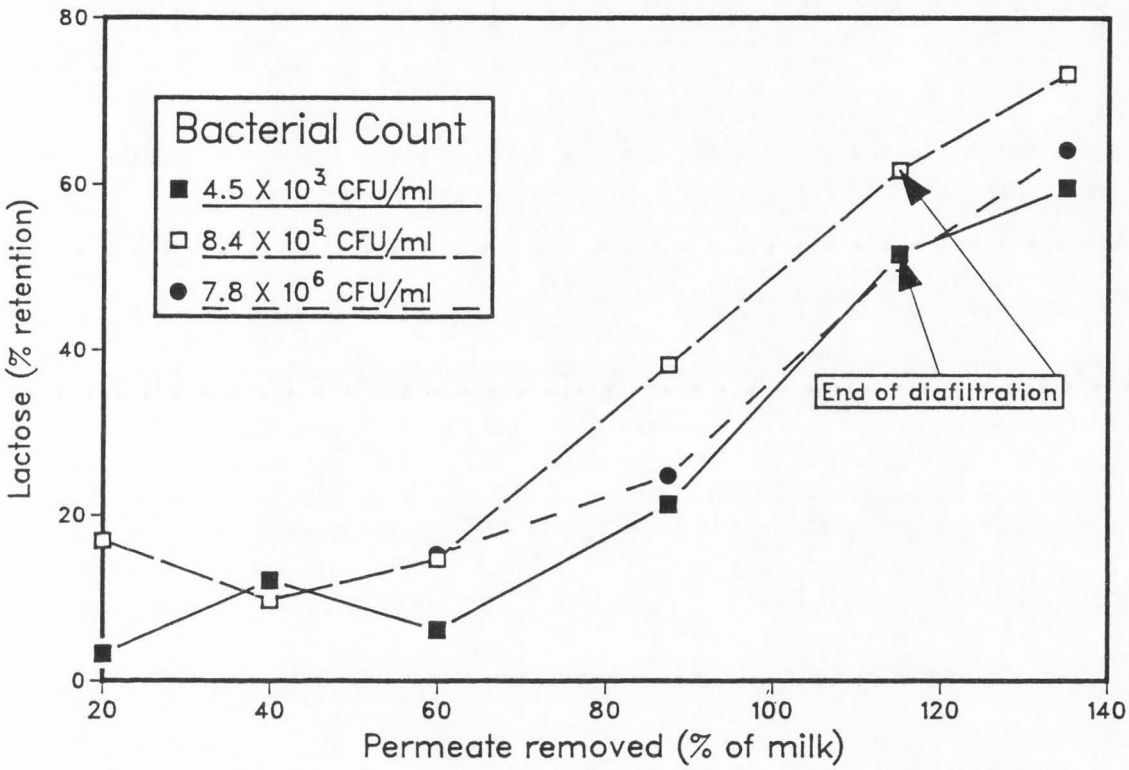


Figure 7. Retention of lactose in milk with high initial bacterial loads.

the other treatments in final lactose concentration. Trends in lactose concentration or recovery in retentate were probably not because of bacteriological quality.

Total solids, protein, fat, and rennet clottable nitrogen were all lower in milk with the highest bacterial count (Figures 1, 2, 8, 9). Since solids was lower, protein, fat, and rennet clottable nitrogen are part of the solids fraction, and likely to be lower. Percent retention of NPN, lactose and protein increased as milk was concentrated by UF (Figures 5, 6, 10). This is typical for most nutrients when diafiltration is used (7). Fenton-May et al. (31) noted that rejection (equivalent to retention) of non-protein constituents of skimmilk was greater than for constituents of whey. Greater rejection for skimmilk was attributed to mass transfer being affected by the casein boundary layer. This boundary layer increased during UF and could be responsible for increased rejection as retentate was concentrated. For retention to increase during UF and diafiltration means there is a greater concentration of nutrient on the retentate side of the membrane than in the permeate. This does not mean that more nutrient is being recovered in the retentate as UF and diafiltration proceeds. Recovery is included (Table 3) so one is not misled to believe that all nutrients with high percent retention have a high recovery.

When milk with high initial bacterial loads was subjected to pressures and temperatures typical of UF processes, the manufacturing process proceeded normally. Differences in final concentration of nutrients in retentate were primarily because of differences in initial concentration in the milk.

Varying Beginning Point of Diafiltration

Process Efficiency. The amount of diafiltration water and procedure for adding water during diafiltration will affect final lactose and salt concentrations in retentate. Starting diafiltration at 60% VR was chosen arbitrarily by Ernstrom et. al. (29), and was used by Bastian (7) and Brown (10). We varied the point at which diafiltration began and adjusted diafiltration water in order to have a final pH of 5.1 to 5.2 after complete

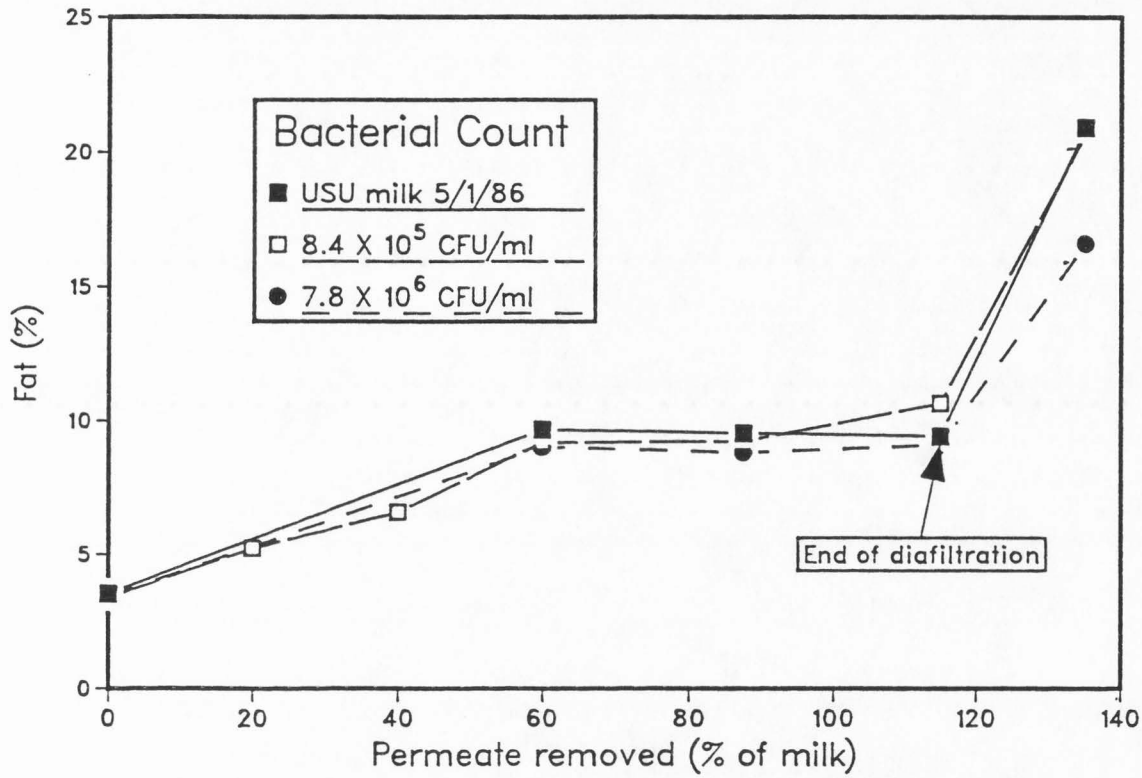


Figure 8. Concentration of fat during UF of milk with high initial bacterial loads.

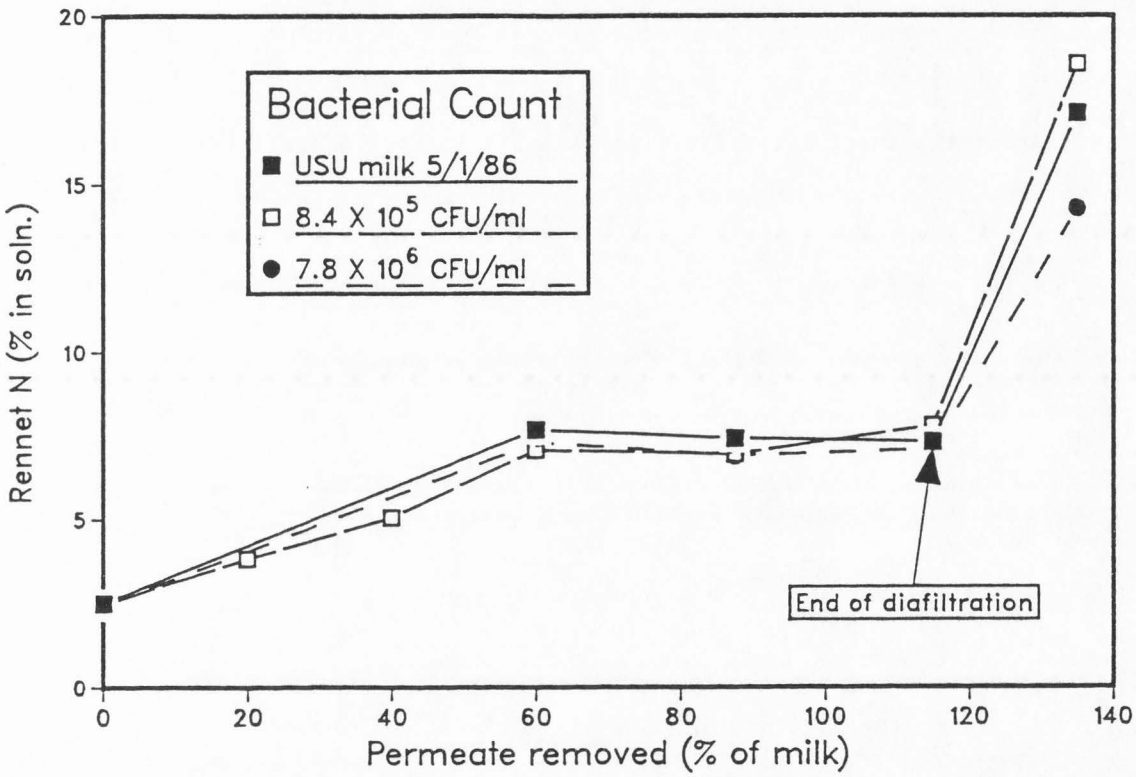


Figure 9. Concentration of rennet clottable nitrogen during UF of milk with high initial bacterial loads.

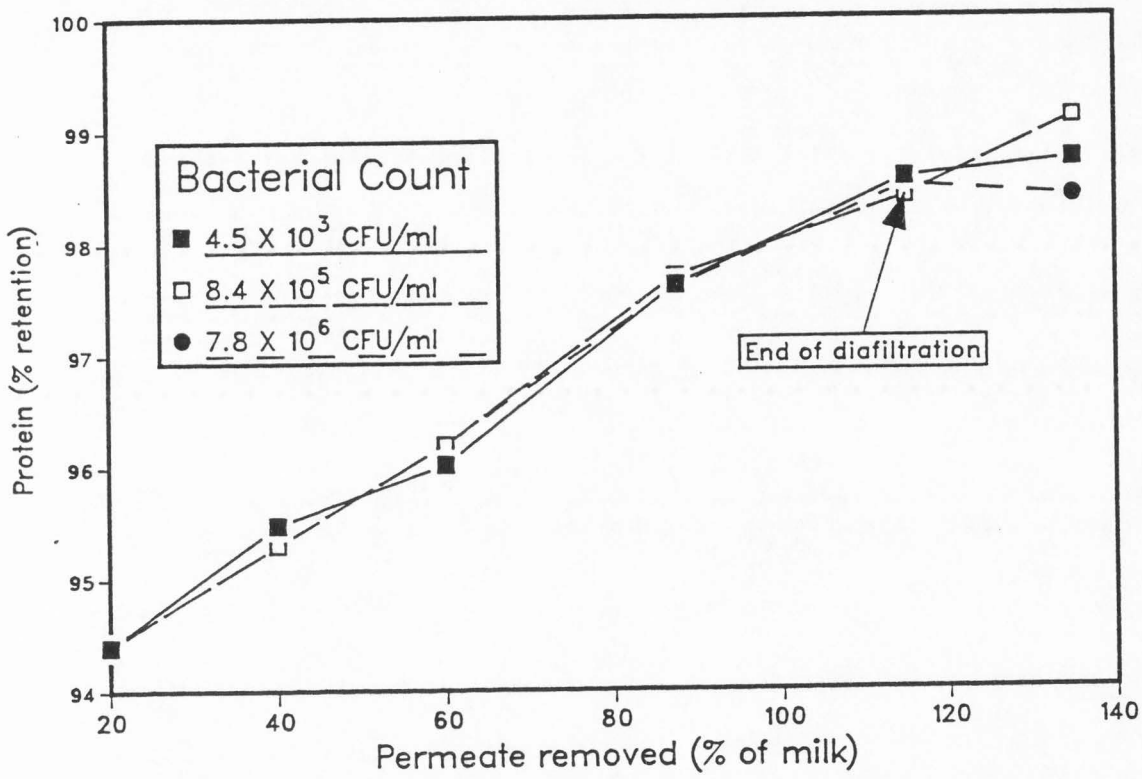


Figure 10. Retention of protein during UF of milk with high initial bacterial loads.

fermentation of the lactose in the final 5× retentate. Diafiltration water was reduced when diafiltration was started after the milk weight was first reduced by 65 or 70% compared to 60%. When diafiltration was started after 60% VR, diafiltration water equaled 55% of the original milk weight. When concentrated to 65% or 70% VR before diafiltration, deionized water for diafiltration was 50% and 38.5% of the original milk weight, respectively. Amount of diafiltration water necessary to reduce lactose is directly dependent on the amount of retentate present during diafiltration. This was observed by Peri et al. (66) and is shown by the direct proportionality between diafiltration water used, D, and mass of water and lactose in the feed tank, M.

$$D = \frac{M}{1 - R} \quad (\ln x_1 - \ln x_2)$$

$$R = \text{retention coefficient of lactose, } 1 - \frac{\text{conc in permeate}}{\text{conc in retentate}}$$

x_1 = mass of lactose at beginning of diafiltration

x_2 = mass of lactose after diafiltration

When diafiltration is begun at a later stage of concentration, there is less mass, M, in the feed tank and less diafiltration water, D, is needed.

An analysis was done to compare total time required for UF compared to water usage when diafiltration was started at 60%, 65%, or 75% VR. Since total volume of milk was different for the three treatments, total time has been calculated for UF of 150 L milk. Reciprocal of permeation rate in hr/L was plotted vs permeate volume in liters (Figure 11). Total time was calculated by determining the area under the three curves. Reciprocal of permeation rate was calculated for a 1 m^2 membrane filtering surface. We were using a membrane with 5 m^2 of filtering surface so final time values were divided by five to give actual times for UF in our system. Table 5 contains a summary of total time and water usage.

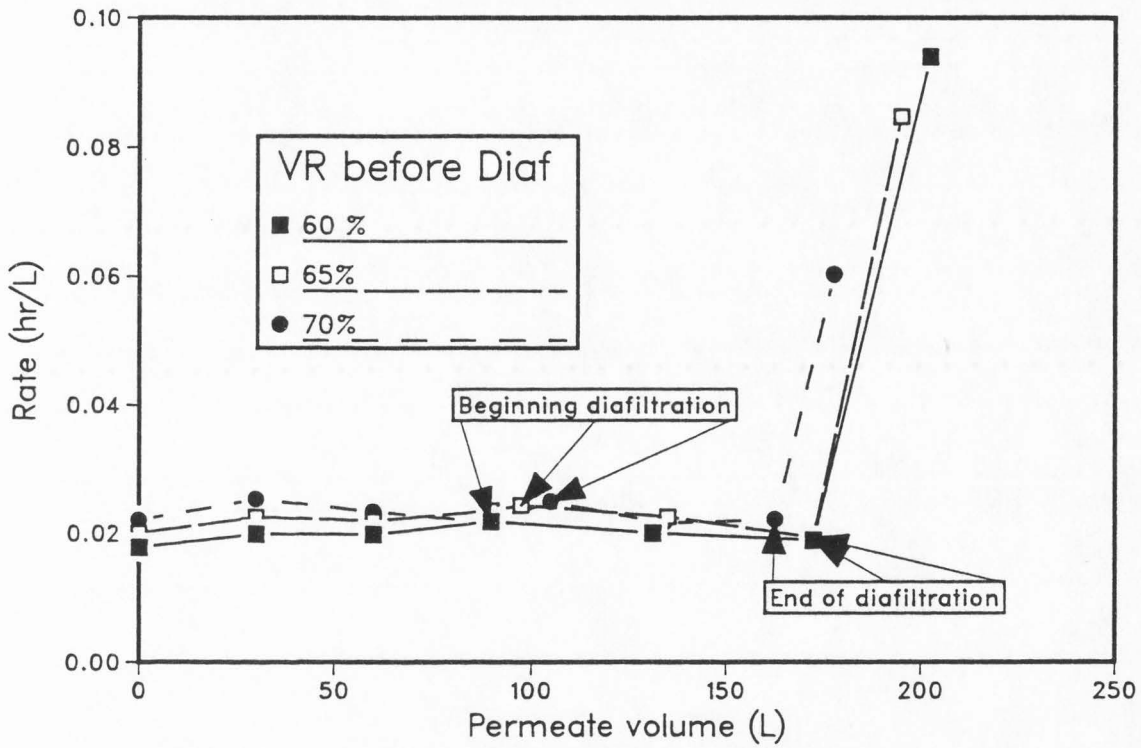


Figure 11. Flow rate during UF in hr/L versus liters of permeate for three different beginning points of diafiltration.

Table 5. Comparison of process time, water usage, and post-fermentation pH for UF of 150 L milk while varying beginning point of diafiltration.

	60% VR	65% VR	70% VR
Total time (hr)	1.029	.999	.875
% of 60% VR time	100	97.1	85.2
Water used (kg)	85	77.3	59.5
% of 60% VR water	100	90.9	70
Post-ferment pH	5.14	5.18	5.19

The target pH of 5.1 to 5.2 was achieved for all three treatments (Table 5). Diafiltration at 65% VR required only 90.9% of the water and 97.1% of the time used for diafiltration at 60% VR. Only 70% of the water and 85.2% of time required for diafiltration at 60% VR were necessary when diafiltration was started at 70% VR. Savings when diafiltration began at 65% VR may be of slight advantage, but time and water savings when diafiltration was started at 70% VR could significantly benefit a manufacturer. These process advantages by beginning diafiltration at 70% VR would be useful if retentate quality and nutrient recovery were as good as that obtained from diafiltration at 60% VR.

Peri et al. (66) determined that total time for UF and diafiltration of skimmilk was a minimum if diafiltration began when protein concentration was 5%. From results of the current study, total time for UF and diafiltration of whole milk was a minimum when protein concentration was 10%.

Nutritional Analysis. Concentrations of protein, fat and total solids were lower in retentate when diafiltration began at 70% VR (Table 6, Figures 12-14). These constituents were lower because the actual concentration factor when calculated as CF(fat) was slightly less for the 70% VR treatment (Table 6). Concentrations of solids, fat and protein (Figures 12-14) during UF were similar for all three treatments. The obvious difference among treatments was because of diafiltration beginning at different points in

Table 6. Nutrient recovery and concentration of nutrients in UF retentate with diafiltration beginning at various stages of volume reduction.

	Volume Reduction Before Diafiltration					
	60%		65%		70%	
Nutrient	Conc	Recov(%)	Conc	Recov(%)	Conc	Recov(%)
Fat (%)	20.55[a]	100	20.80[b]	100	19.91[c]	100
Protein(%)	15.02[a]	92.6	14.85[a]	90.5	14.29[b]	90.9
Ren N(%TN)	77.44	95.4	81.55	98.1	80.1	96.8
Ion Ca (%)	.06[a]	36.7	.049[b]	28.3	.045[c]	27.1
Lactose(%)	1.47[a]	5.8	1.60[a]	6.3	1.88[b]	7.7
Buffer Capac[d]	11.80		11.58		11.54	
Ca(%)	.296[a]	64.4	.275[b]	59.2	.294[a]	66.1
P(%)	.235	47.9	.229	46.3	.232	48.8
Rib(μg/g)	.193	7.2	.184	6.6	.177	10.3
B_{12}(ng/g)	10.36	65.1	10.49	65.1	9.71	62.9
Solids(%)	38.01[a]	56.7	37.11[b]	54.7	36.79[c]	56.6
CF(fat)	5.404		5.47		5.237	

[abc]For a particular nutrient, means with the same letter are not significantly different at α=.01 using Fishers protected LSD.

[d]meq HCl/100 g sample.

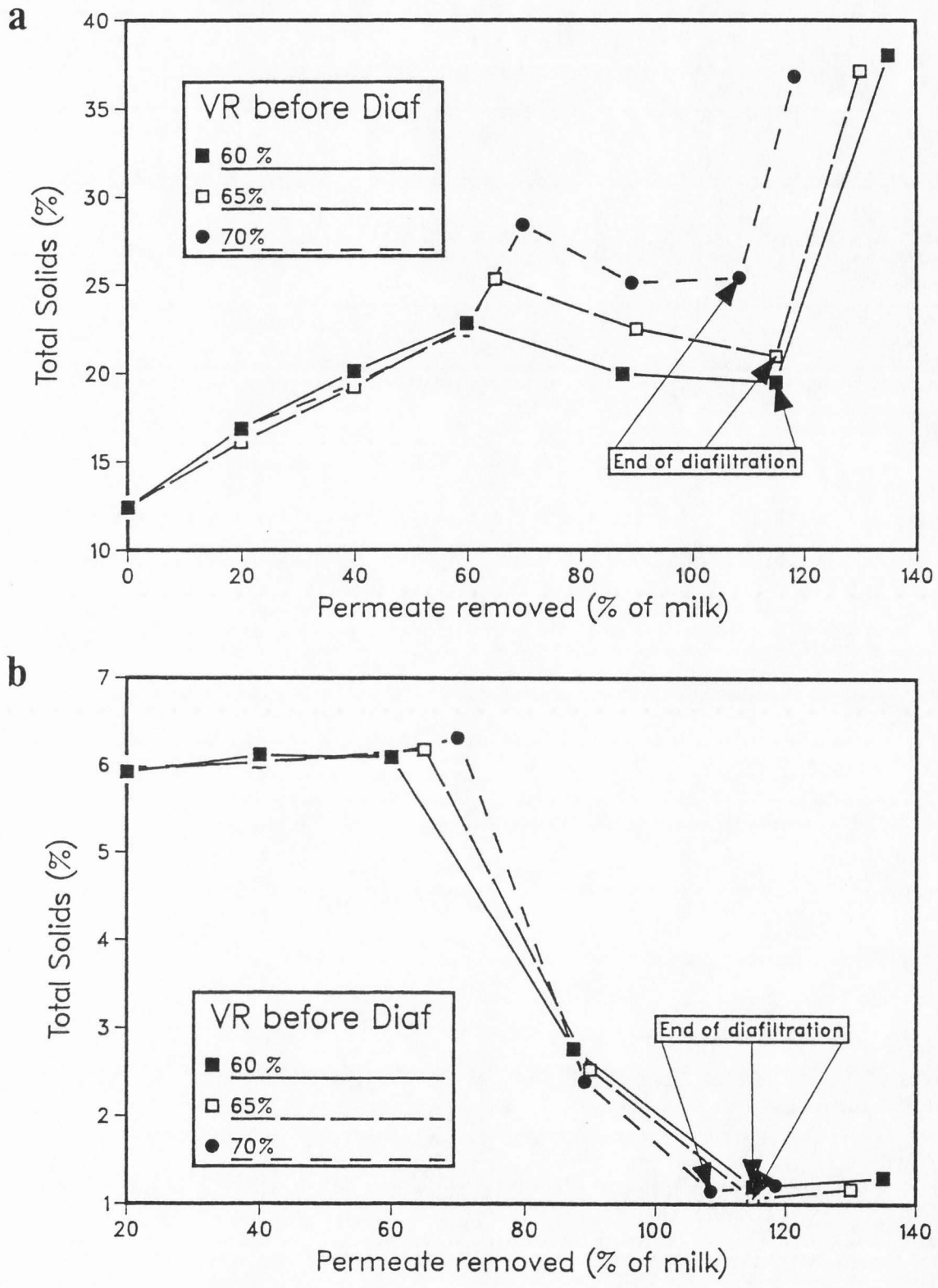


Figure 12. Total solids in (a) retentate and (b) permeate while varying beginning point of diafiltration.

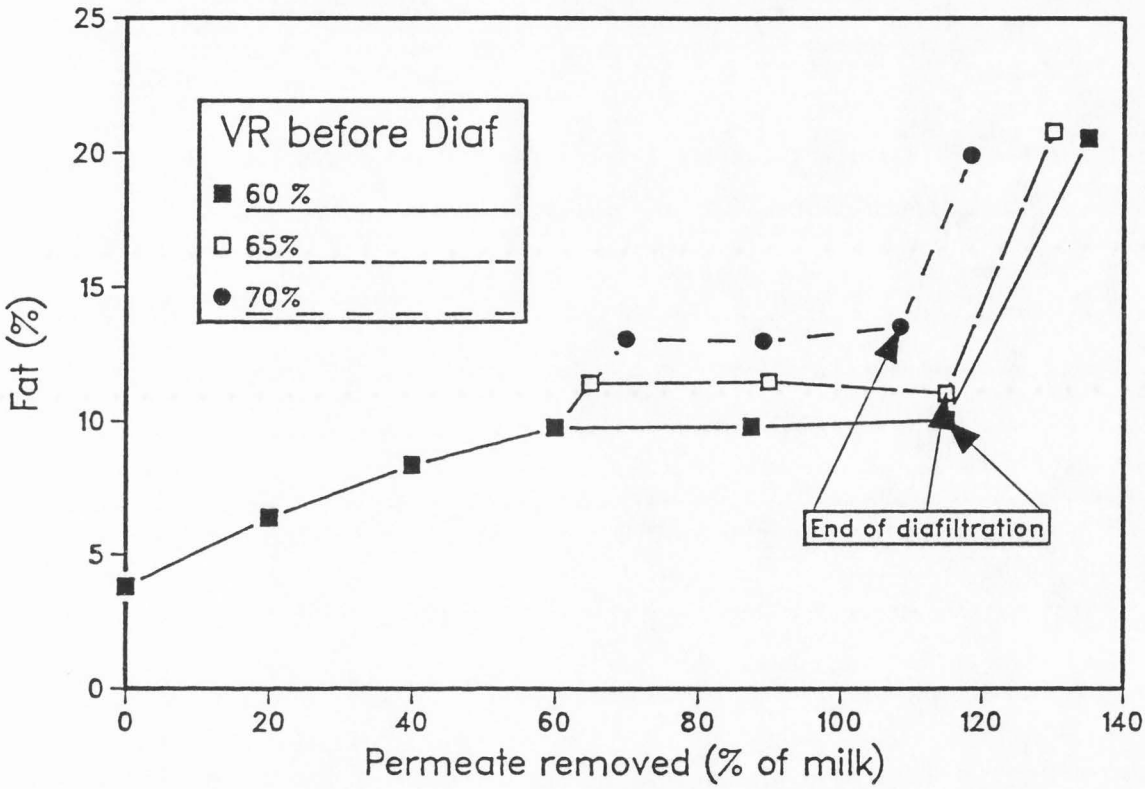


Figure 13. Fat concentration in retentate while varying beginning point of diafiltration.

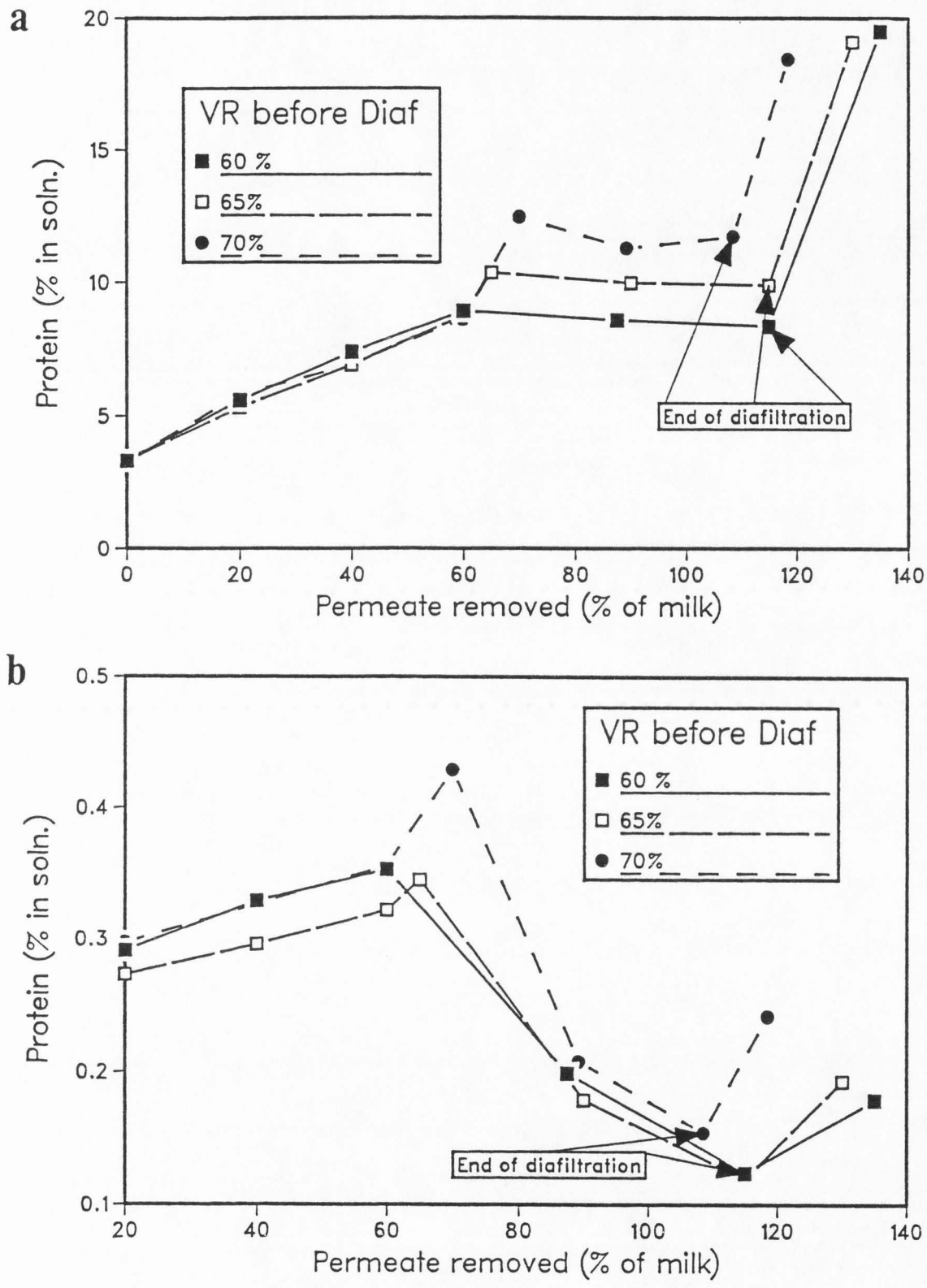


Figure 14. Protein concentration in (a) retentate and (b) permeate while varying beginning point of diafiltration.

the concentration process. Hence, solids, fat, and protein were at three distinct levels during diafiltration.

Percent retention of protein increased during UF and diafiltration (Figure 15), much like it did in the milk quality study. There were no apparent differences among treatments. Rennet clottable nitrogen, as percent of total nitrogen, was slightly lower when diafiltration began at 60% VR (Table 6). Rennet clottable nitrogen, as percent in solution, was similar for all treatments (Figure 16), and changed like total protein (Figure 14) during UF and diafiltration. Lactose concentration and percent retention were higher in the sample that was diafiltered least, 70% VR (Figures 17,18). This was expected since less lactose was washed out when less diafiltration water was used. With the exception of calcium and ionic calcium, there were no other significant differences in nutrient content of the retentates (Table 6). Final ionic calcium concentration was greatest in the 60% VR and lowest in the 70% VR sample (Table 6, Figure 19). Throughout diafiltration, ionic calcium concentration and retention (Figures 19, 20) were lower in retentate that was diafiltered more and higher when less water was used. It was expected that increased diafiltration water would decrease concentration of ionic calcium in retentate as was observed by Bastian (7). Only at the 5× concentration was ionic calcium greater in the treatment with more diafiltration, 60% VR. Retention of ionic calcium during UF was highest in the treatment that was diafiltered the least, 70% VR (Figure 20), as would be expected. Final concentration of Ca and P was only slightly different (Table 6, Figures 21, 22), although when diafiltration began at 65% VR, Ca concentration in retentate was significantly lower.

Retention of these minerals was slightly higher in the 70% VR treatment (Figures 23 and 24). Minerals such as Ca and P are not free to pass through the membrane like lactose, and are affected less by amount of diafiltration water. Unlike lactose, Ca and P are both strongly associated with the casein micelles as colloidal calcium phosphate. When diafiltration was started at 60% VR, increased diafiltration did not decrease Ca and

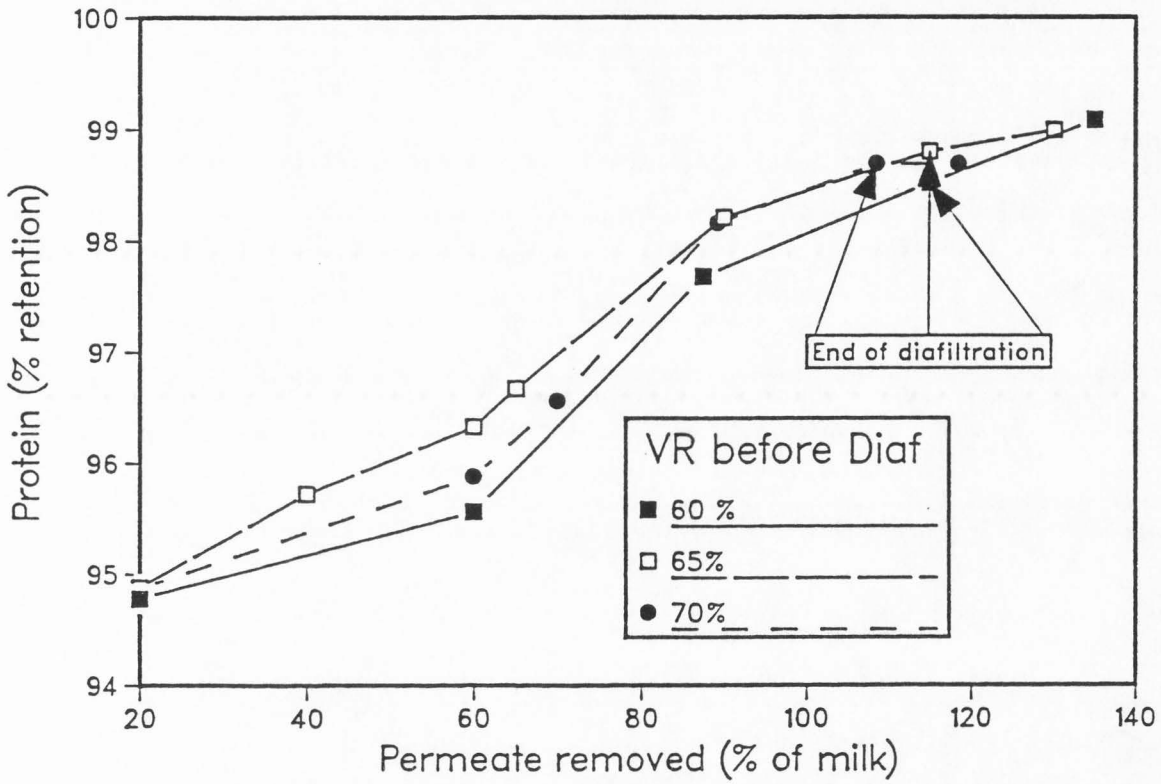


Figure 15. Retention of protein while varying beginning point of diafiltration.

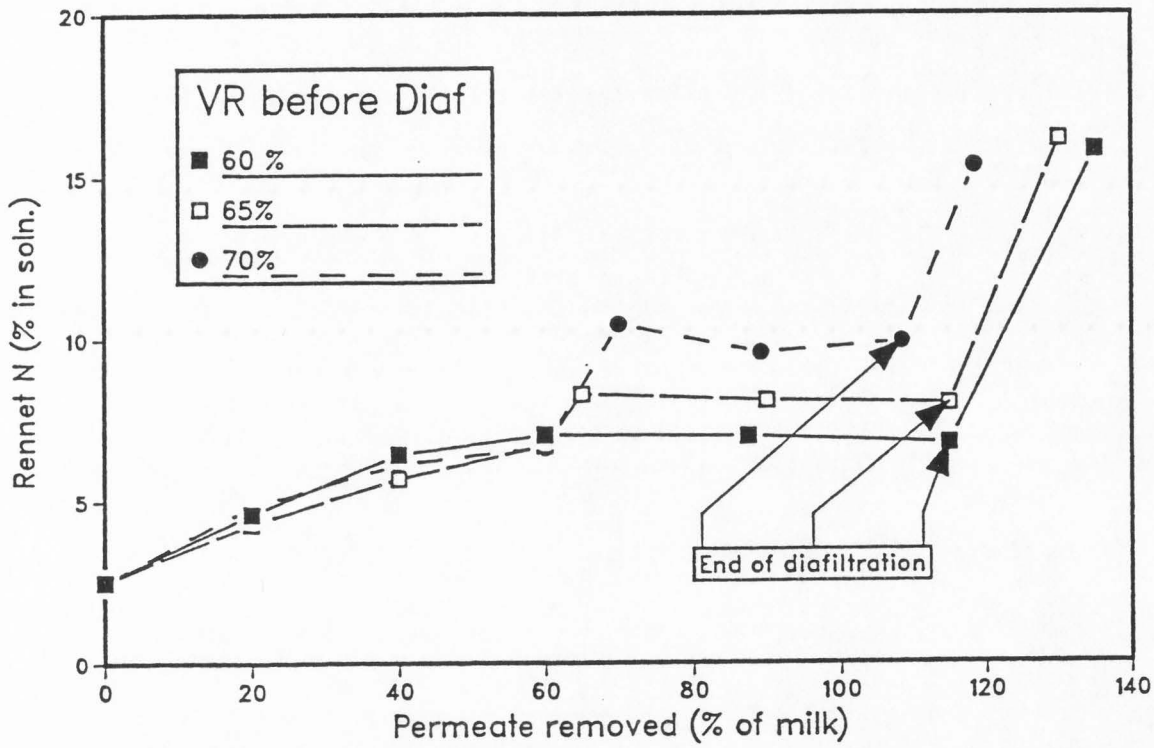


Figure 16. Concentration of rennet clottable nitrogen while varying beginning point of diafiltration.

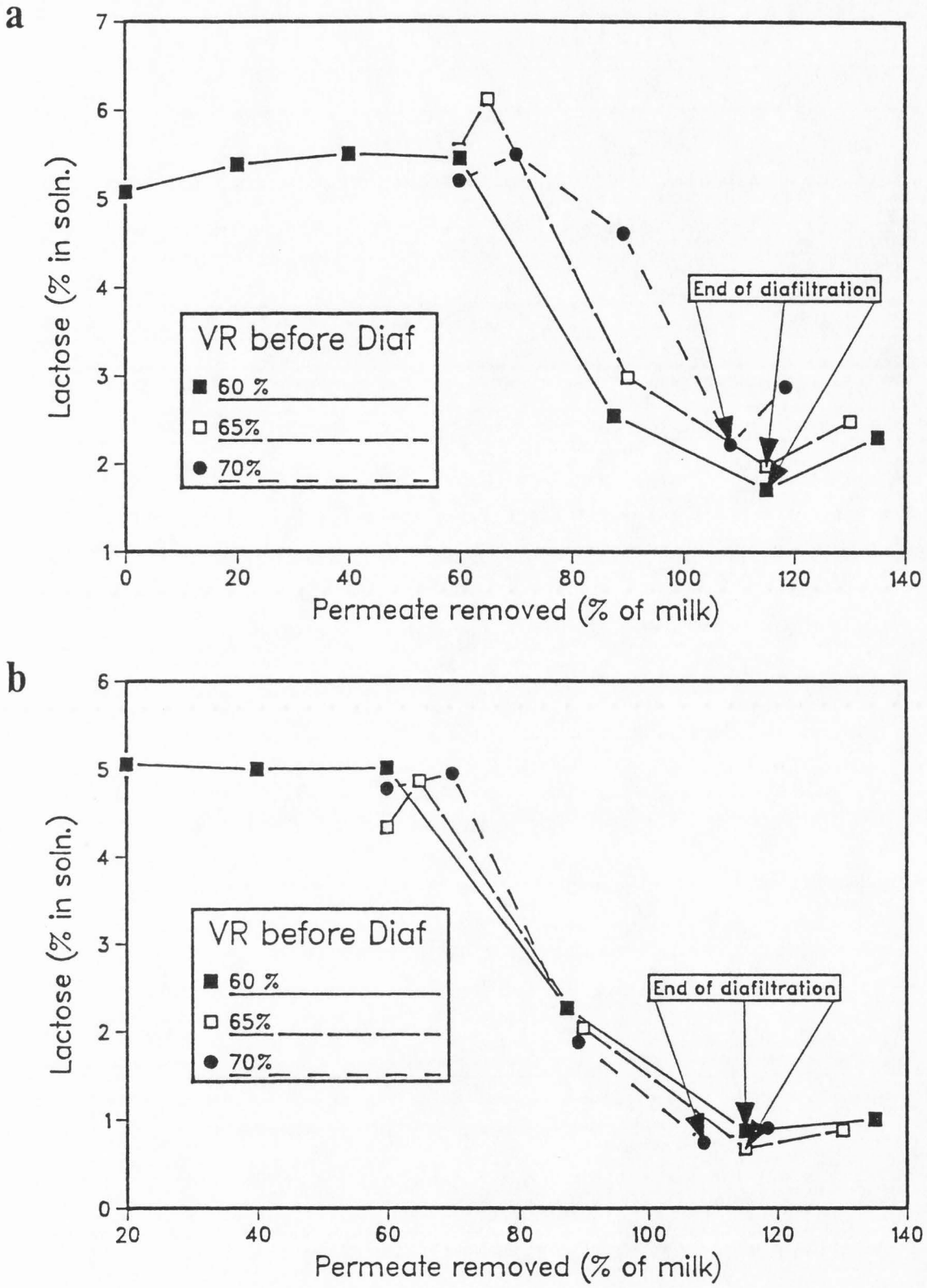


Figure 17. Lactose concentration in (a) retentate and (b) permeate while varying beginning point of diafiltration.

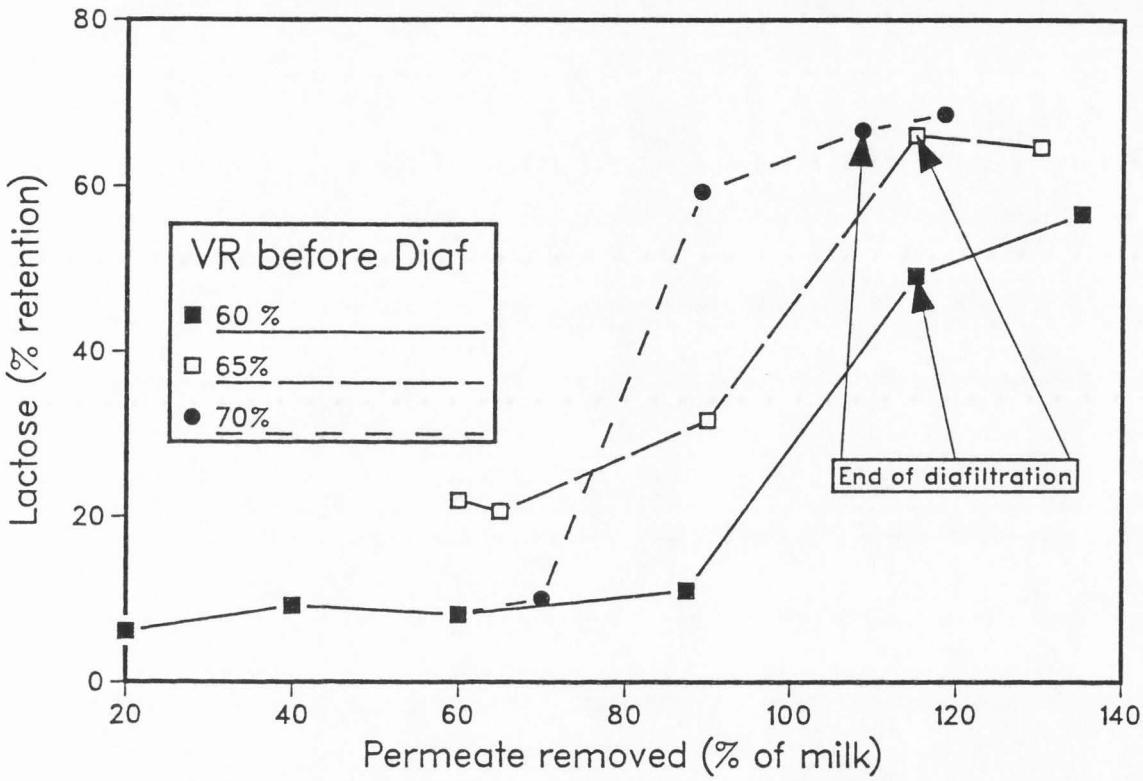


Figure 18. Retention of lactose while varying beginning point of diafiltration.

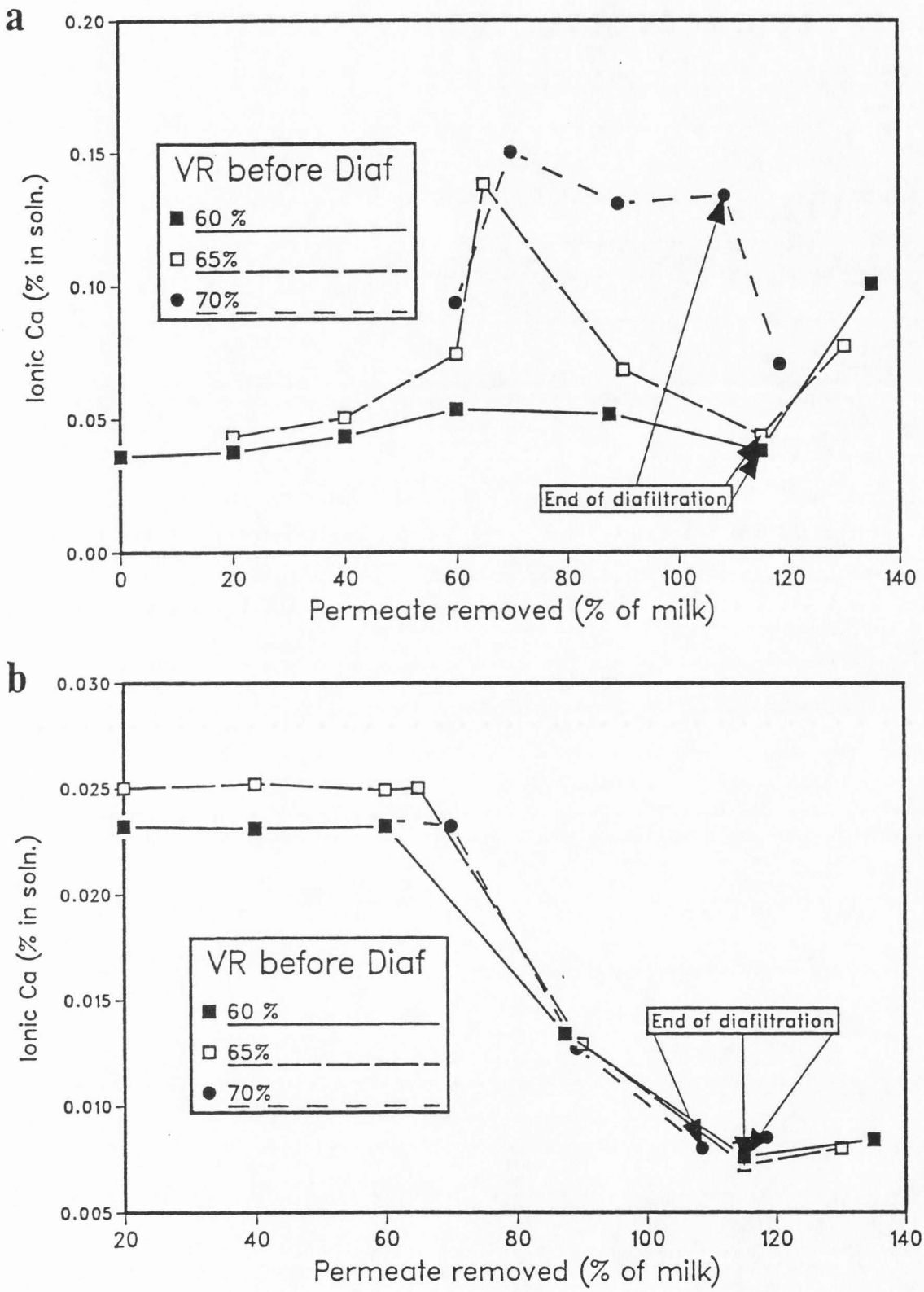


Figure 19. Ionic calcium concentration in (a) retentate and (b) permeate while varying beginning point of diafiltration.

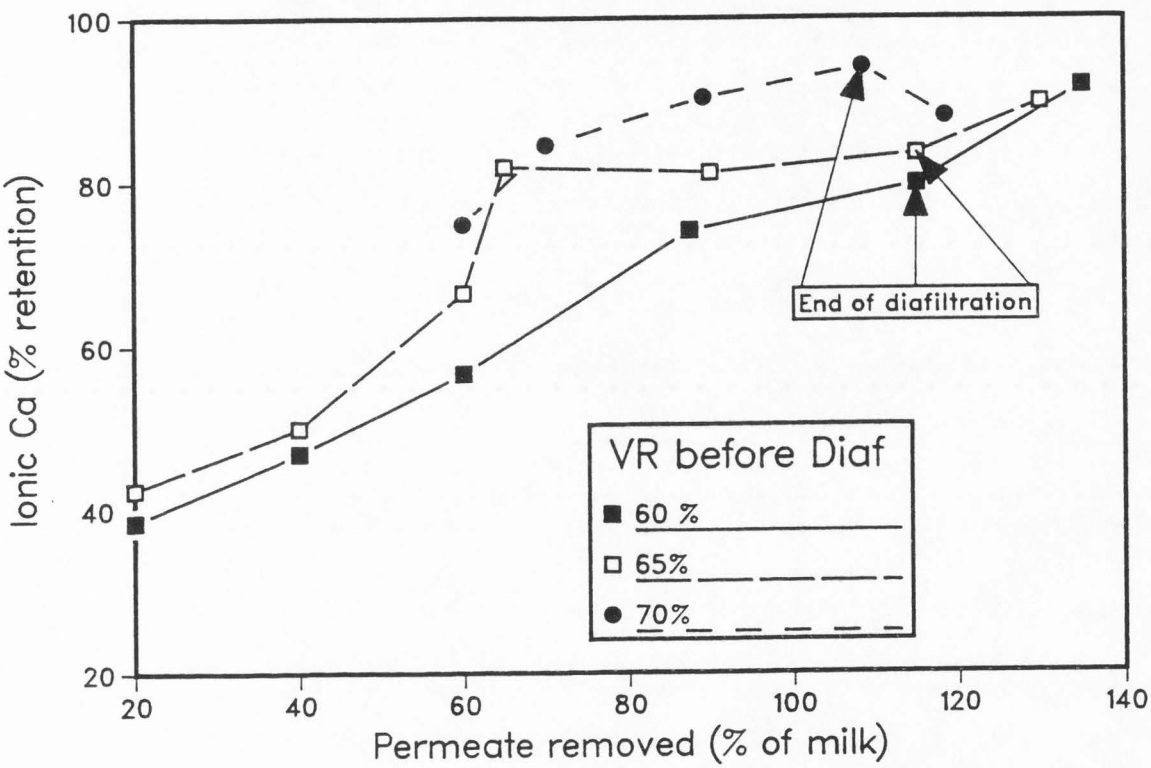


Figure 20. Retention of ionic calcium while varying beginning point of diafiltration.

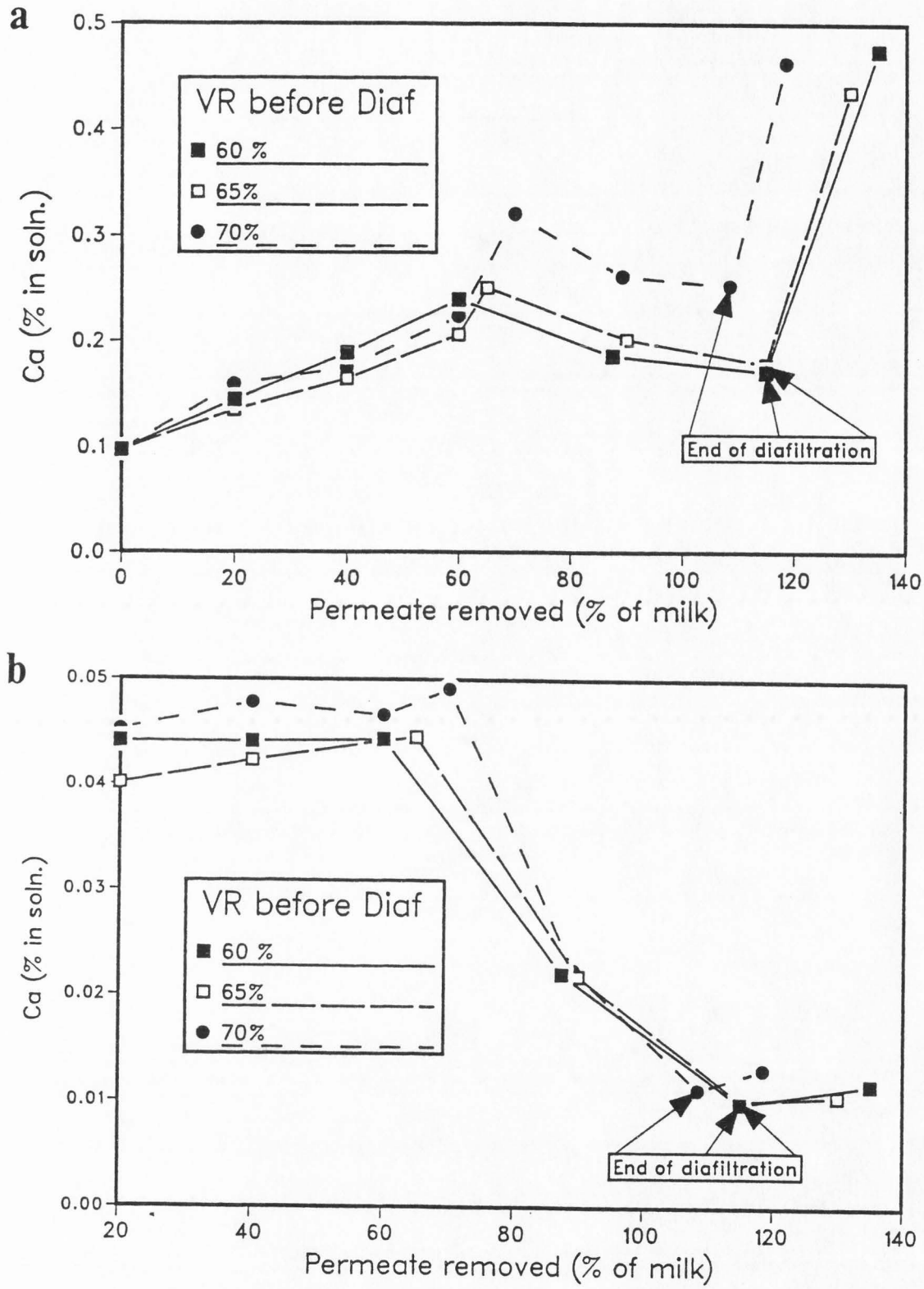


Figure 21. Concentration of calcium in (a) retentate and (b) permeate while varying beginning point of diafiltration.

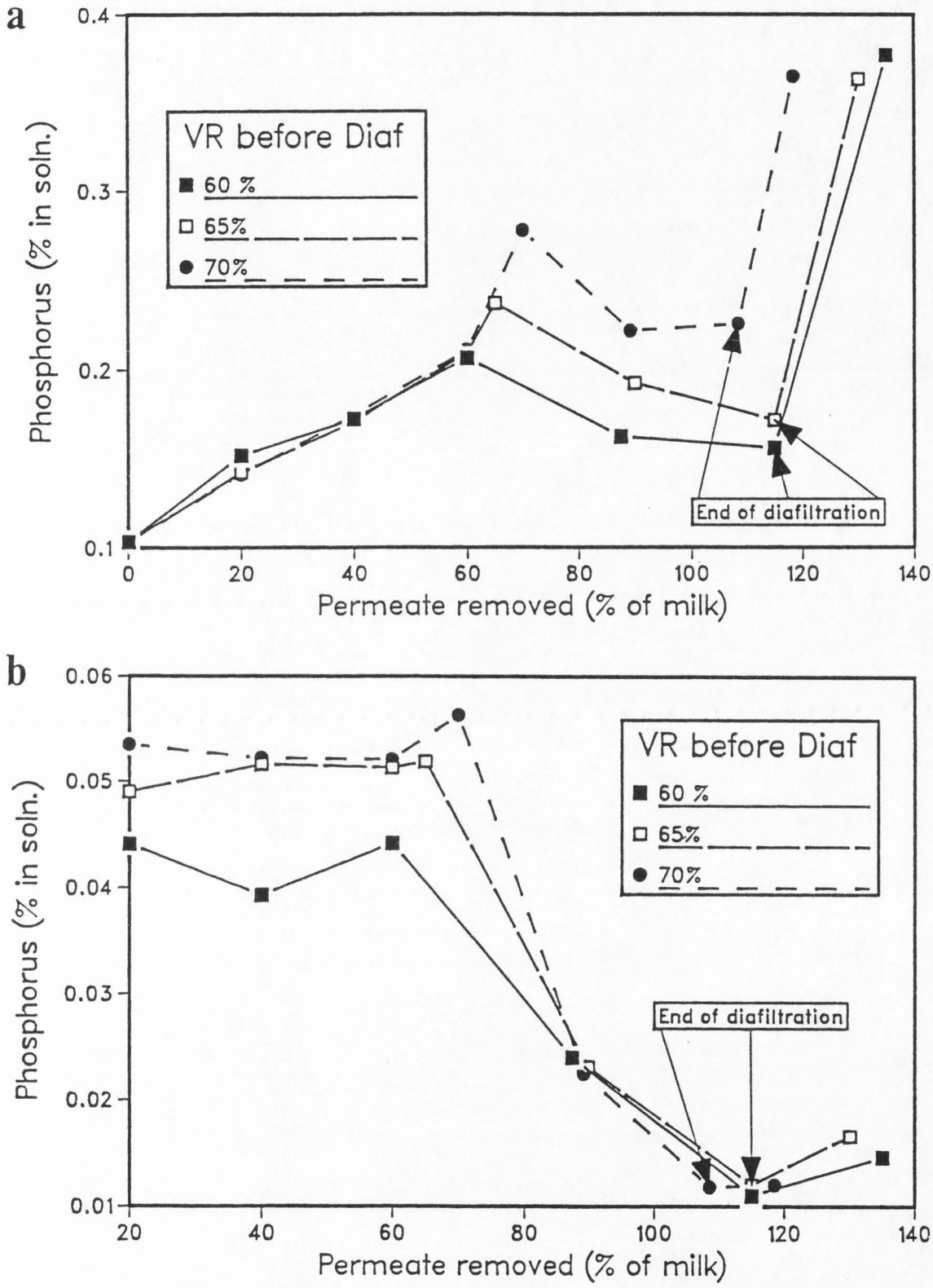


Figure 22. Concentration of phosphorus in (a) retentate and (b) permeate while varying beginning point of diafiltration.

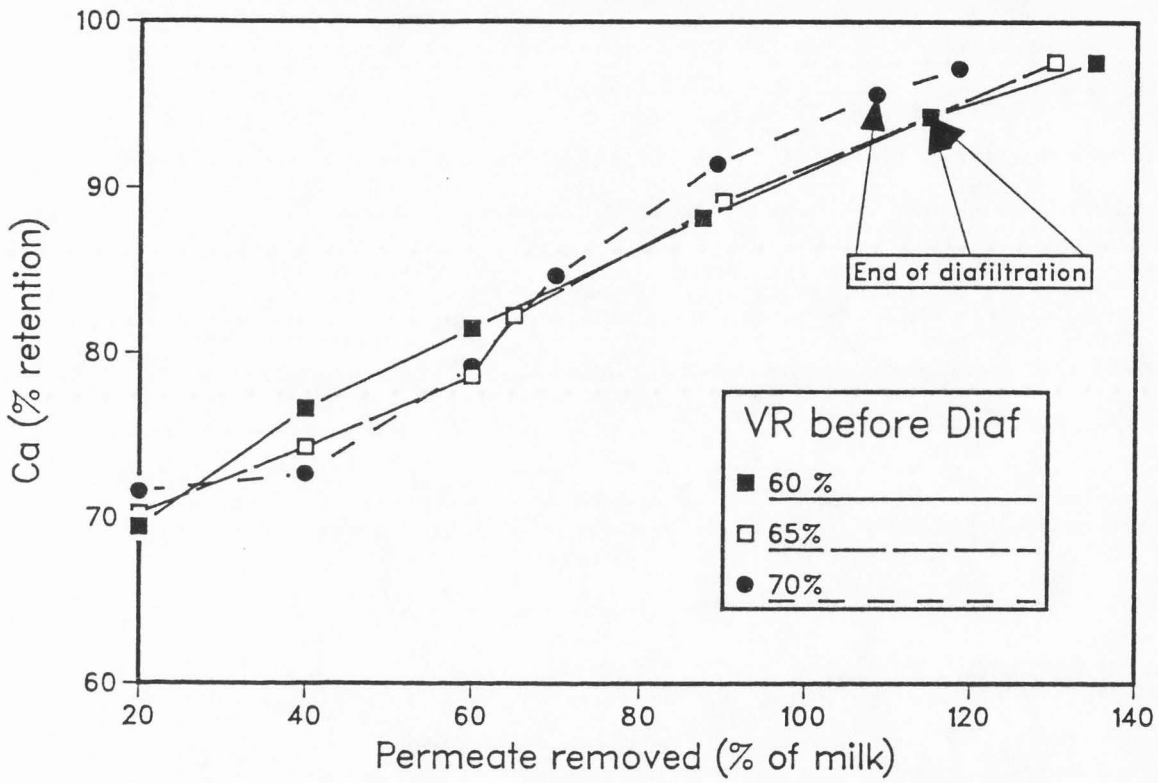


Figure 23. Retention of calcium while varying beginning point of diafiltration.

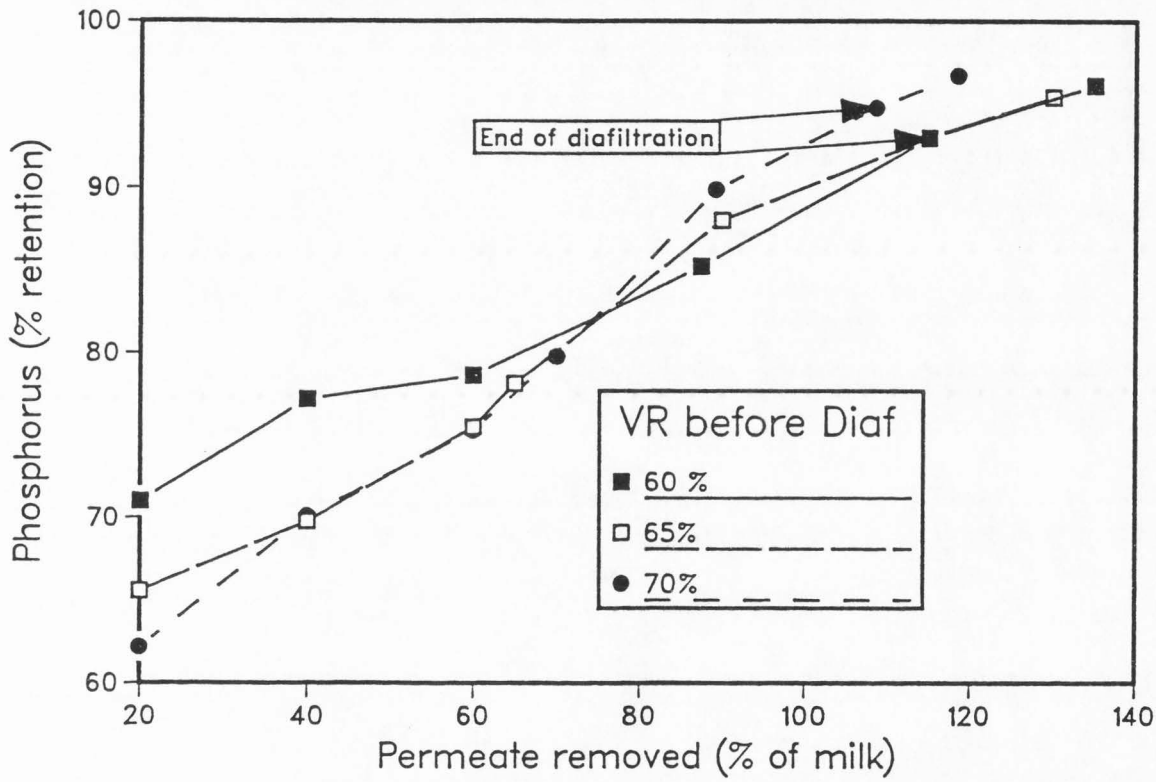


Figure 24. Retention of phosphorus while varying beginning point of diafiltration.

P in the 5× retentate (7). Riboflavin concentration and retention were similar for all treatments but concentration was slightly higher in retentate with the most diafiltration (Figures 25 and 26). As with ionic calcium, Bastian (7) reported less riboflavin with increased diafiltration. Concentration of vitamin B_{12} in retentate was not significantly different (Table 6). When diafiltration began at 60% VR, permeate contained more vitamin B_{12} (Figure 27) and percent retention decreased (Figure 28). Slightly more vitamin B_{12} permeated the membrane when diafiltration began at 60% VR. Vitamin B_{12} is bound to protein and had retentions of 86-98 percent (Figure 28). Buffer capacity for the three treatments was not significantly different (Table 6). Brown (10) found no significant difference in buffer capacity when diafiltration level varied from 35-80 percent of initial milk weight.

Permeate contains mainly lactose, salts, and water soluble vitamins. Concentration of all these constituents decreased during diafiltration and then increased slightly following diafiltration (Figures 14b, 17b, 19b, 21b, 22b, 25b, 27b). Decrease in concentration was because of dilution with water during diafiltration. Total solids concentration decreased as would be expected from loss of these nutrients (Figure 12b).

Diafiltration at 70% VR took less time and water than diafiltration at 60 or 65% VR. Nutrient quality and recovery were not reduced. Optimum diafiltration could be performed by beginning after 70% of the initial milk weight was removed.

Heat Treatment and Process Cheese Food Meltability

Study A: Acidification and Heat Treatment Prior to UF. During the first part of this study, Study A, all pasteurized process cheese food was cooked to 82°C. Meltability was similar for all three pH groups. The average melt distance for process cheese food prepared from UF of unacidified milk was 17 mm and 9 mm for cheese from milk acidified to pH 5.8 (Figure 29). Previously, Ernstrom and Anis (28) showed improved meltability in process cheese food made from preacidified ultrafiltered milk. Using the

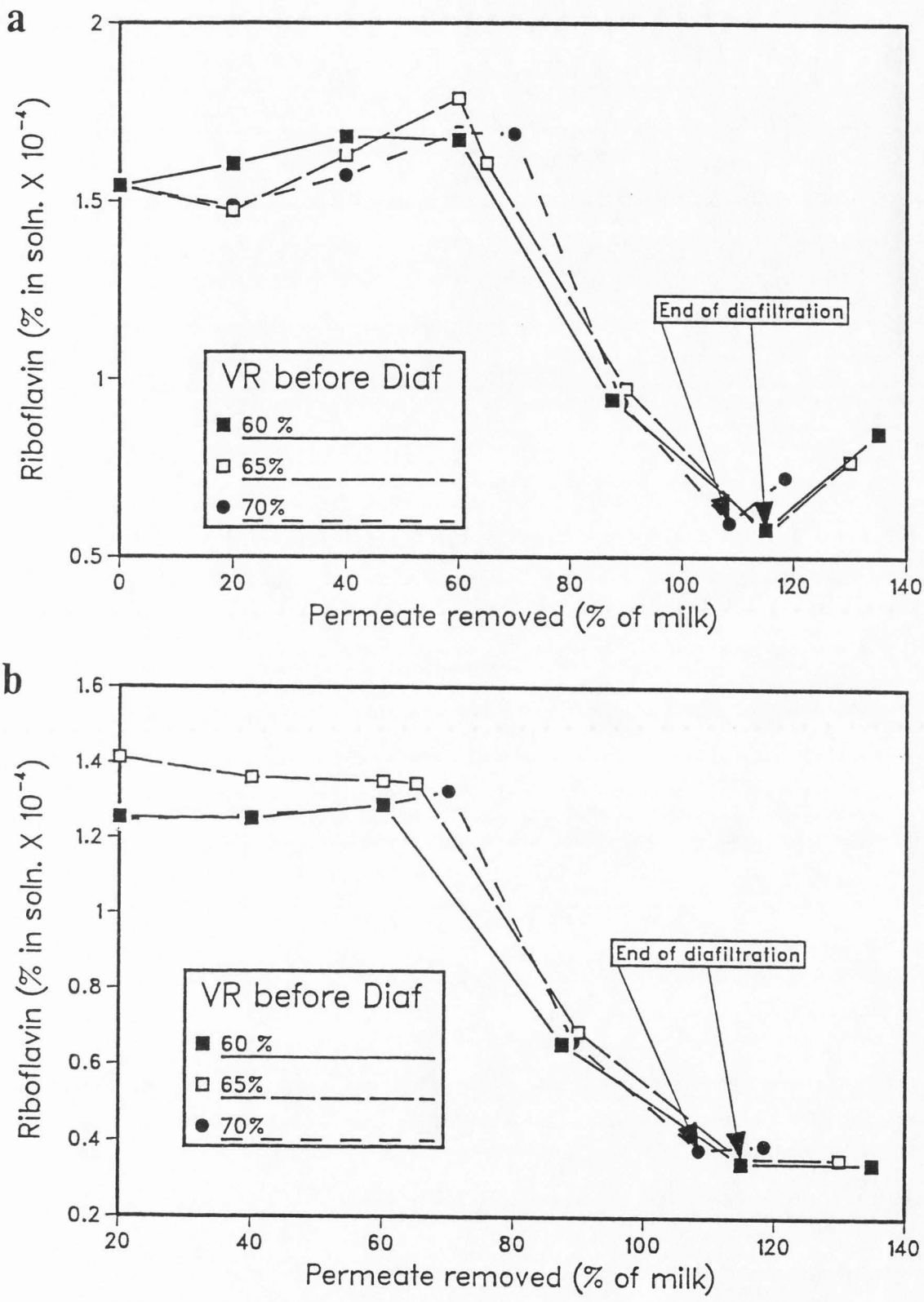


Figure 25. Riboflavin concentration in (a) retentate and (b) permeate while varying beginning point of diafiltration.

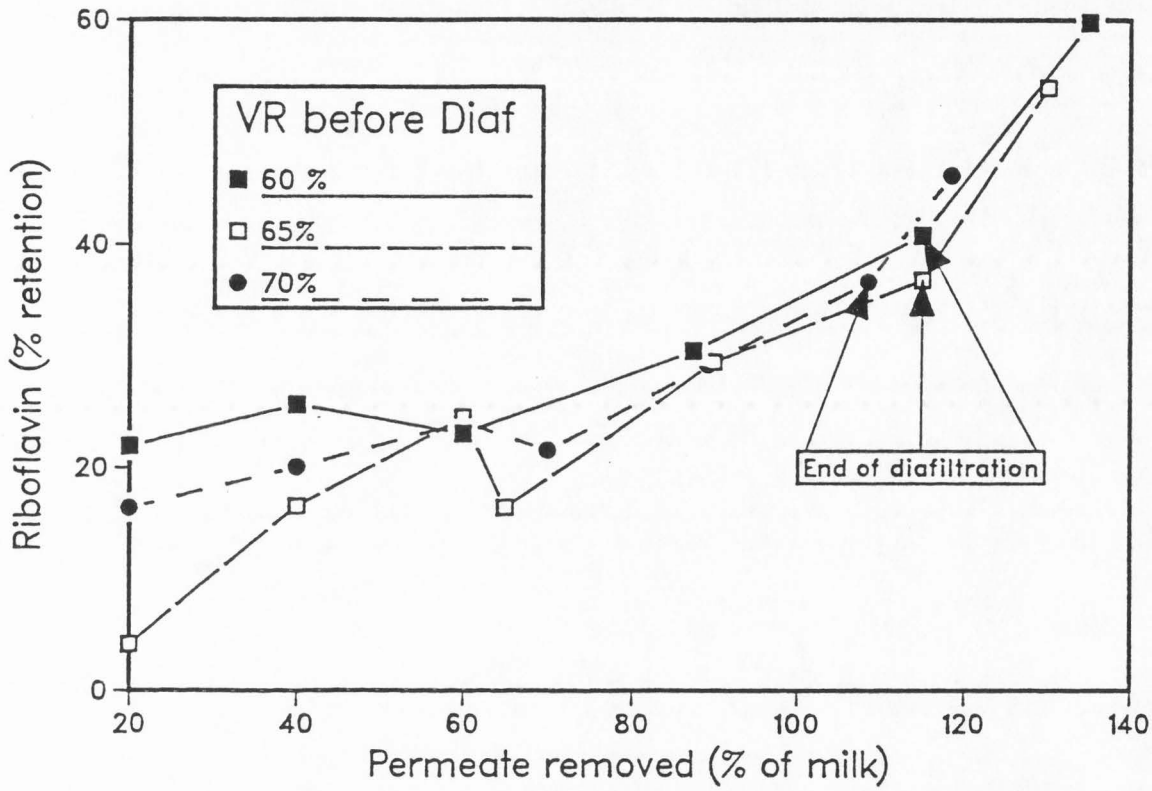


Figure 26. Retention of riboflavin while varying beginning point of diafiltration.

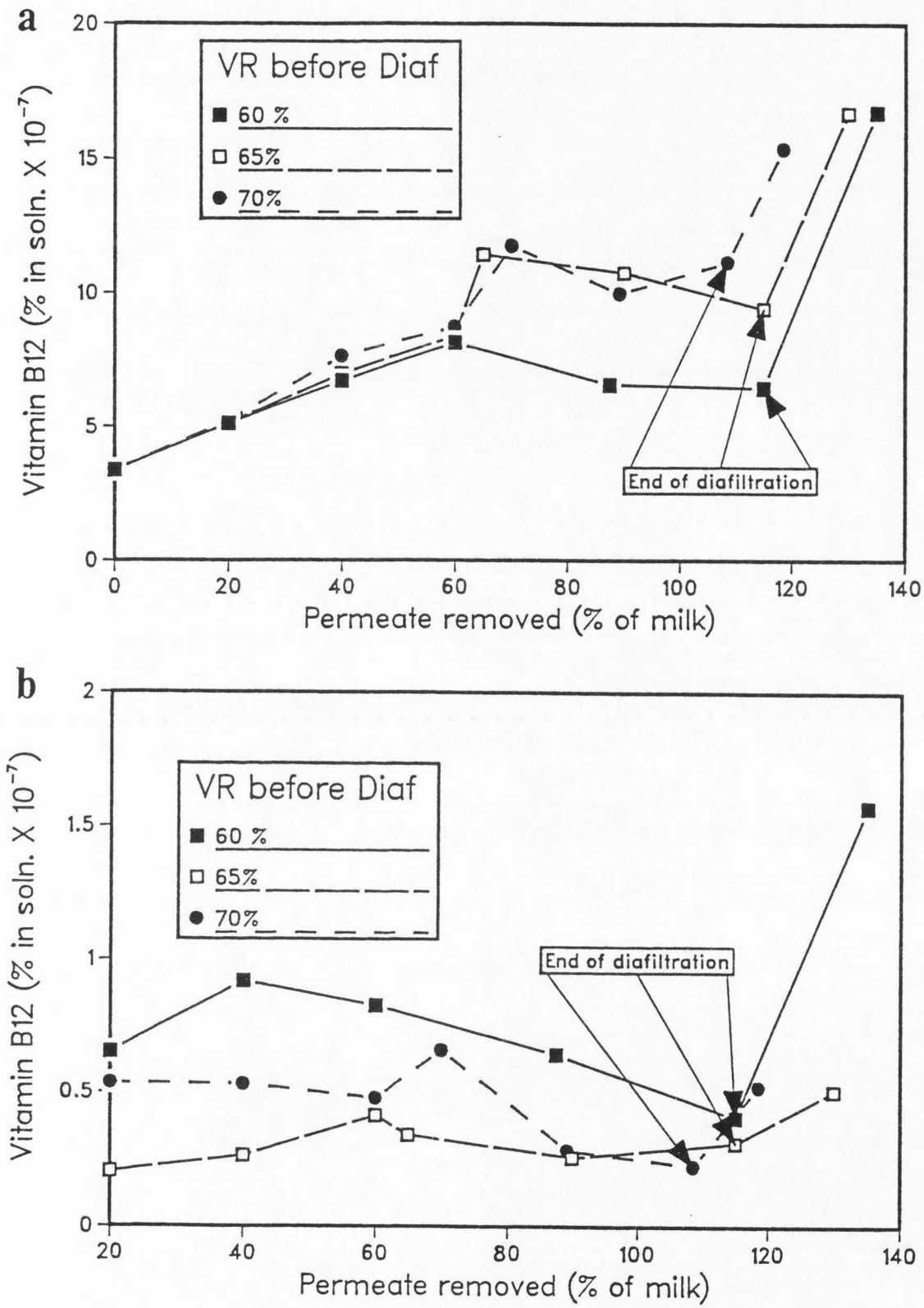


Figure 27. Vitamin B_{12} in (a) retentate and (b) permeate while varying beginning point of diafiltration.

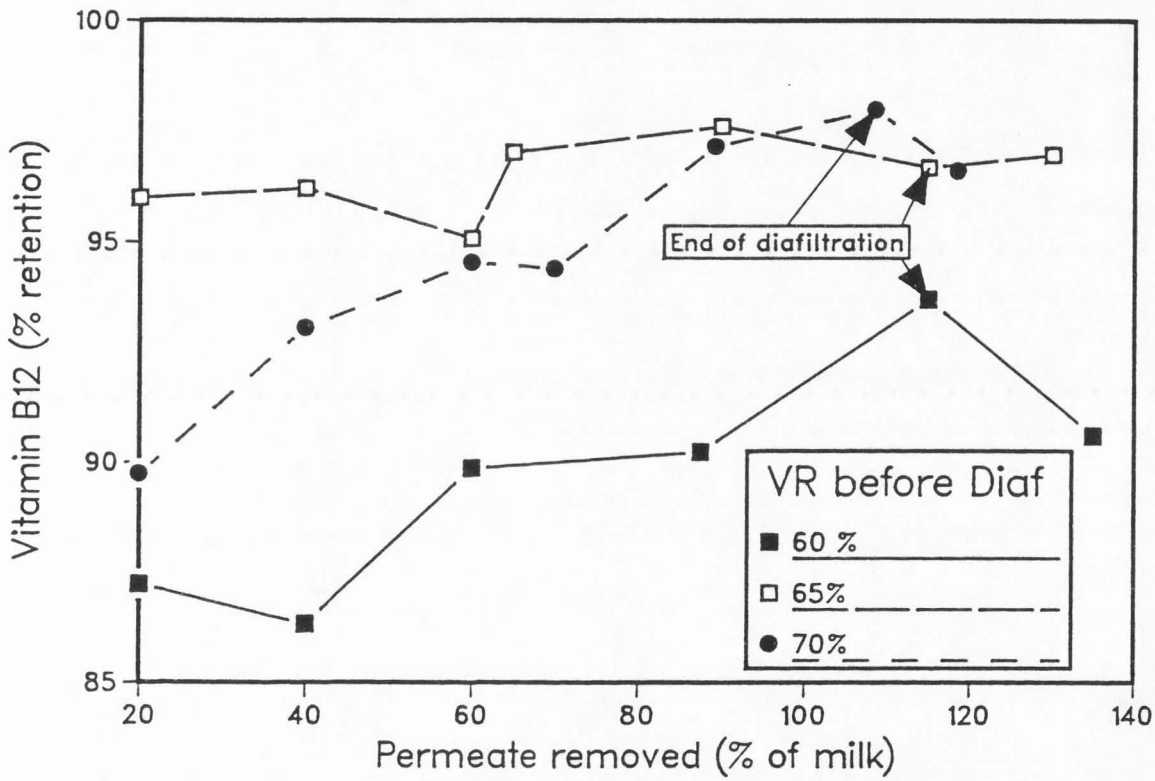


Figure 28. Retention of vitamin B_{12} while varying beginning point of diafiltration.

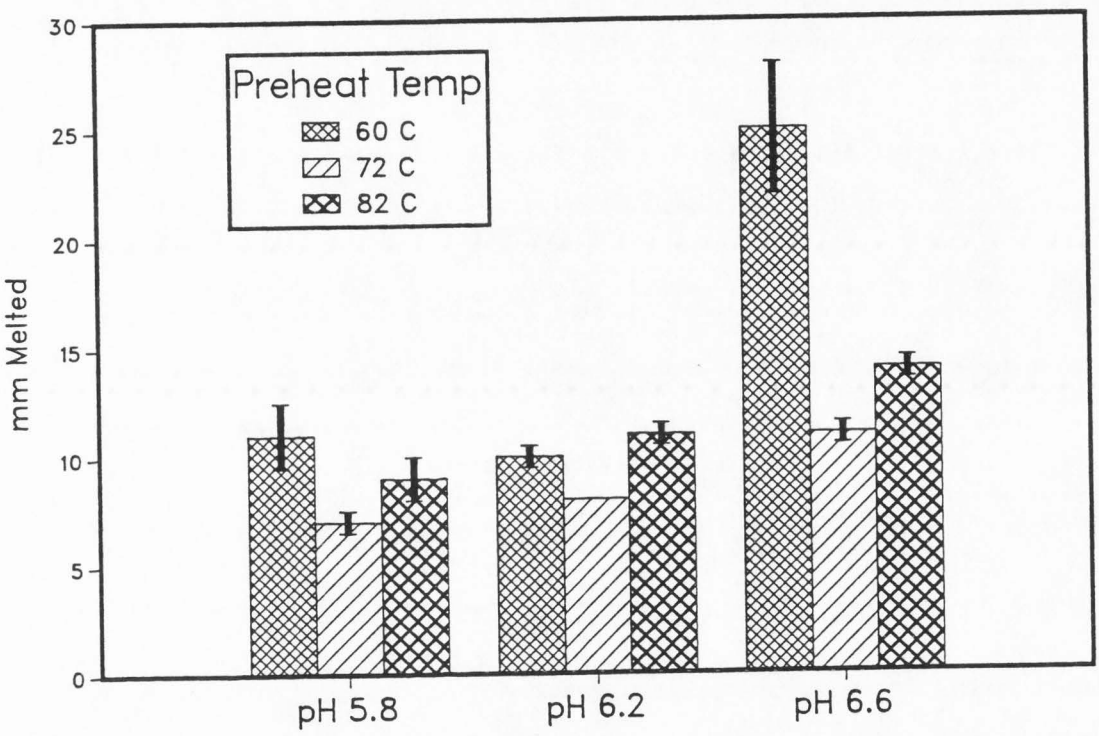


Figure 29. Meltability of pasteurized process cheese food cooked to 82°C. Milk was preheated to 60, 72, or 82°C for 16 s. Error bars represent standard error of the mean.

exact method to evaluate meltability, they reported approximately 2 mm melt distance for unacidified milk and 65 mm for cheese made from milk acidified to pH 5.8.

Study B: Effect of Preheating, Acidification, and Cooking Temperature. To verify the results of Study A, the experiment was repeated, Study B, using more diafiltration water and three cooking temperatures. Again, cheese made from preacidified ultrafiltered milk did not melt as well as cheese from unacidified milk (Figure 30). Meltability was evaluated for three levels each of pH, preheat temperature, and cook temperature. Least squares ANOVA was used to analyze the data because there was one missing data point. The experiment was designed as a 3^3 factorial with three subsamples for each treatment. It was not replicated so there was no true estimate of experimental error, although subsampling error was included in the error term to perform the ANOVA. Cooking temperature significantly affected meltability (Table 7). Cheese cooked to 70°C had the

Table 7. Analysis of variance of preheat, pH, and cooking temperature on meltability of pasteurized process cheese food.

Source	df	Sums of Squares	F ratio	Significant α
Preheat temperature	2	198.6	1.67	.1983
Cook temperature	2	27720.8	232.92	.0001
pH	2	3397.6	28.55	.0001
Preheat × cook	4	1706.3	7.17	.0001
Preheat × pH	4	343.3	1.44	.2329
Cook × pH	4	149.9	.63	.6433
Preheat × pH × cook	8	1249.1	2.62	.0169
Error	53	3153.8		
Corrected Total	79	37501.95		

best meltability at all pH levels. The main effect of preheating was insignificant although the preheat by cook interaction was significant (Appendix E). Both preheating the milk and cooking the cheese affect protein denaturation and might interact to affect meltability.

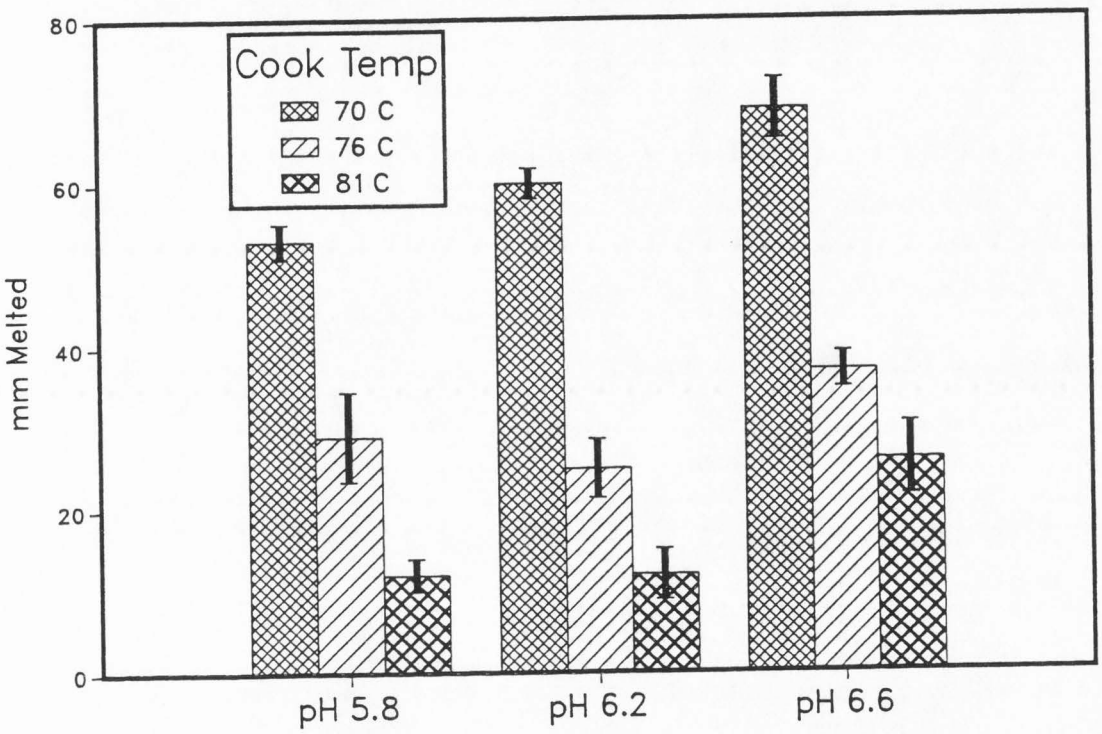


Figure 30. Effect of cooking temperature on meltability of pasteurized process cheese food after preacidifying milk to pH 5.8, 6.2, or 6.6. Error bars represent standard error of the mean.

Cheese made from unacidified ultrafiltered milk (pH 6.6) melted better than cheese made from milk acidified to pH 6.2 or 5.8 before UF.

Rayan (71) investigated the effect of heat treatment on meltability of process cheese made with natural Cheddar cheese. He showed a decrease in meltability with increased cooking time from 0 to 30 min at 82°C. Increased cooking time would affect the overall heat treatment of the cheese and could decrease meltability in the same way higher temperature affects process cheese food made from UF.

Whey powder may be added to process cheese food made by conventional methods. Savello (72) felt that undenatured whey proteins in UF retentate may be the cause of poor meltability. In model process cheese, inclusion of either denatured or undenatured whey protein caused similar decreased meltability. Decreased meltability in UF process cheese could be because complexes form between whey protein and casein during cooking. Functional properties of casein in UF retentate could be different than casein in Cheddar cheese for processing.

For some uses of process cheese it is desirable to prevent melting. Schulz (77) patented a method for manufacturing process cheese that is resistant to melting. Addition of albumin or globulin proteins prior to cooking process cheese produces a melt-resistant product. Typical albumins or globulins would be α-lactalbumin or β-lactoglobulin found in whey. It is likely that heat denaturation of these proteins is responsible for the poor melting ability of UF process cheese food.

When disodium oxalate was added to cheese made with TSPP or DSP, meltability was greatly improved (72). Savello felt this was because oxalate is superior to DSP and TSPP in calcium binding ability. Citrate also improved meltability in rennet casein model process cheese more than in the same product made with acid casein. He believed this was because citrate sequesters calcium, which is in greater concentration in rennet casein than acid casein. We also had greater meltability in the sample with the most calcium, in agreement with Savello's work. Citrate is also known to increase the water holding

capacity of protein, and may improve solubility (49). Solubility of casein is improved because sodium citrate increases the pH and sequesters calcium. Both these functions increase the negative charge of casein, and prevent precipitation. If protein solubility were the most critical factor in process cheese meltability, it would be logical for cooking temperature to have a great effect.

Lazaridis and Rosenau (52) reported no improvement in meltability of process cheese made with citrate added at .5 or 1.5%. When 3.0% sodium citrate was added, meltability improved but was less than when trisodium or disodium phosphate were used. In their study, cheeses were cooked at 80°C for 22 to 25 min. Meltability results may have been different if products were cooked for less time at a lower temperature.

During manufacture of mozzarella made by direct acidification (76), preheating milk to 80, 90, 100, 110, 120, or 130°C for 2 s did not significantly affect meltability. They reported a 3.4% increase in cheese yield because of recovery of denatured whey protein in the curd. We also found no significant differences in meltability when milk was subjected to a short heat treatment.

Study C: Effect of Heating Retentate Before Manufacture of Process Cheese Food. Since all four heat treatments were from one batch of retentate, nutrient values were the same for all cheeses. Melting ability was best when retentate was preheated to 61°C and cheese was cooked to 70°C (Figure 31). Preheating retentate to 72 or 83°C before process cheese manufacture significantly decreased meltability (Table 8). Cooking temperature had the greatest effect on meltability. All preheat and cook temperatures had significantly different effects at $\alpha = .01$. This experiment was not replicated and subsamples were considered as replicates for analysis purposes.

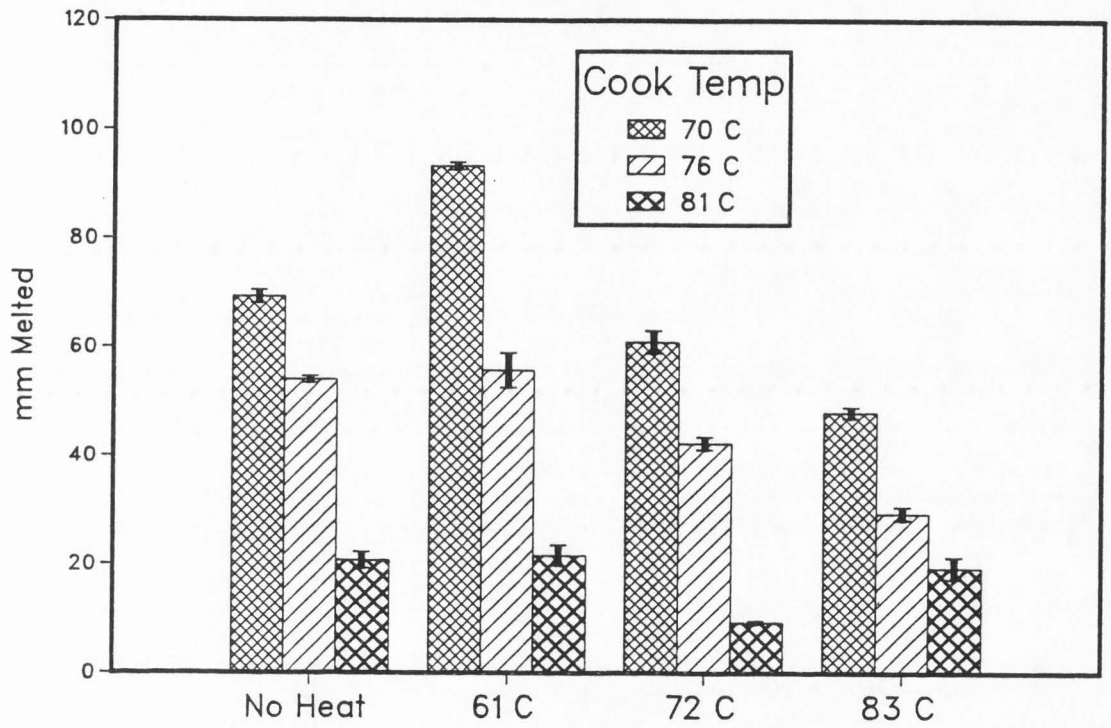


Figure 31. Effect of heating retentate for 16 s and cooking temperature on meltability of pasteurized process cheese food. Error bars represent standard error of the mean.

Table 8. Analysis of variance of heat treatment of retentate and cooking temperature on meltability of process cheese food.

Source	df	Sums of Squares	F ratio	Significant α
Heat treatment	3	3257.3	150.4	.0001
Cook temperature	2	15150.2	1049.2	.0001
Heat × Cook	6	1652.5	338.15	.0001
Error	24	182		
Corrected Total	35	20242		

Nutrient Recovery. To evaluate the effect of preheating milk on nutrient recovery in 5× retentate, analysis of variance was performed using recovery data from study A and B (Table 9). Recovery of solids, protein, calcium, and phosphorus was compared for both studies. Riboflavin and lactose data were only available for study A. No differences were found for solids, protein or lactose. Riboflavin was affected both by preheating and acidification (Table 10). Milk with the lowest heat treatment, 60°C, had greater recovery of riboflavin in the retentate than when milk was preheated to 82°C. When preheat temperature was 72°C, recovery was no different from when preheated to 60 or 82°C. After preheating to 82°C, riboflavin was probably released from protein, making it more easily lost during UF and diafiltration. Riboflavin recovery was highest when the milk pH was 6.2 or 6.6 and lowest when acidified to pH 5.8 (Table 10). Milk at pH 6.2 or 6.6 was also diafiltered less than pH 5.8 milk, so decrease in recovery could be due more to diafiltration than acidification. Increased diafiltration is necessary with decreasing pH to keep the final lactose limiting pH of retentate between 5.1 and 5.2. Calcium and phosphorus were also affected by pH and amount of diafiltration. Recovery of Ca and P was less when milk was acidified to pH 5.8 or 6.2.

A comparison of the nutrient composition of process cheese food made from unacidified ultrafiltered milk and Cheddar cheese is included in Table 11. As a percentage

Table 9. Summary of effects of preheating on percent recovery of nutrients in 5× retentate.

	Preheat Temperature		
Constituent	60-61°C	72°C	82-83°C
Solids	57.85[a]	57.33[a]	58.84[a]
Protein	91.77[a]	89.70[a]	91.98[a]
Lactose	5.15[a]	5.45[a]	6.19[a]
Riboflavin[d]	9.27[a]	7.65[ab]	6.68[b]
Calcium	50.97[ab]	48.24[b]	56.35[a]
Phosphorus	52.37[a]	49.46[a]	49.74[a]

[abc]For a particular nutrient, means with the same letter are not significantly different at α=.05 using Fishers protected LSD.

[d]Riboflavin and lactose data are from study A with one replication. Means with the same letter are not significantly different at α = .01 using Fishers protected LSD.

Table 10. Summary of effects of acidification on percent recovery of nutrients in 5× retentate.

	pH		
Constituent	5.8	6.2	6.6
Solids	57.01[a]	58.56[a]	58.45[a]
Protein	90.32[a]	92.05[a]	91.08[a]
Lactose	4.80[a]	5.84[a]	6.16[a]
Riboflavin[d]	5.96[a]	8.30[b]	9.34[b]
Calcium	41.49[a]	52.38[b]	61.69[c]
Phosphorus	43.63[a]	51.44[b]	56.50[c]

[abc]For a particular nutrient, means with the same letter are not significantly different at α=.05 using Fishers protected LSD.

[d]Riboflavin and lactose data are only from study A, with one replication. Means with the same letter are not significantly different at α = .01 using Fishers protected LSD.

Table 11. Comparison of nutrients in UF pasteurized process cheese food and Cheddar cheese. SEM is standard error of the mean. Dry matter includes milk solids and NaCl, excluding the sodium citrate of process cheese food.

Nutrient	UF Cheese Unacidified	% of Dry Matter	Cheddar Cheese	% of Dry Matter
	Mean (SEM)		Mean (SEM)	
Solids(%)	55.0(.019)		61.5(0)	
Protein(%)	20.4(.0005)	38.9	22.8(.125)	37.1
Fat(%)	26.6(.014)	50.7	34.3(.180)	55.8
P(%)	.369(.003)	.70	.448(.003)	.73
Ca(%)	.518(.003)	.99	.617(.001)	1.00
B_{12}(ng/g)	20.47(.230)	3.9×10^{-6}	19.29(.210)	3.14×10^{-6}
Rib(μg/g)	.70(.004)	1.3×10^{-4}	1.54(.090)	2.5×10^{-4}

of the dry matter, percent fat, phosphorus, and calcium were slightly lower in UF process cheese food than in Cheddar cheese. Vitamin B_{12} and protein were slightly higher in UF cheese and the riboflavin content was about half that of Cheddar cheese.

Effect of Cooking Temperature on Meltability and Soluble Nitrogen at pH 4.6

Meltability decreased with increase in cooking temperature (Figure 32). Between 66 and 72°C, decrease in meltability was 9.8 mm. The greatest drop in meltability was between 72 and 76°C, a decrease of 29.2 mm. This was probably because of denaturation of β-lactoglobulin that occurs at 70°C (22). Soluble protein at pH 4.6 is an indicator of the amount of undenatured whey protein in the cheese. Soluble nitrogen decreased with increased cooking temperature (Figure 32) probably because of denaturation of β-lactoglobulin and other milk proteins. By least squares analysis, soluble nitrogen at pH 4.6 was positively correlated with meltability with R=.95 (Figure 33). PAGE was used to evaluate whey proteins that were denatured at higher cooking temperatures. The most obvious changes were loss of bovine serum albumin and β-lactoglobulin in cheese cooked to 82°C, compared to cheese cooked to 66°C

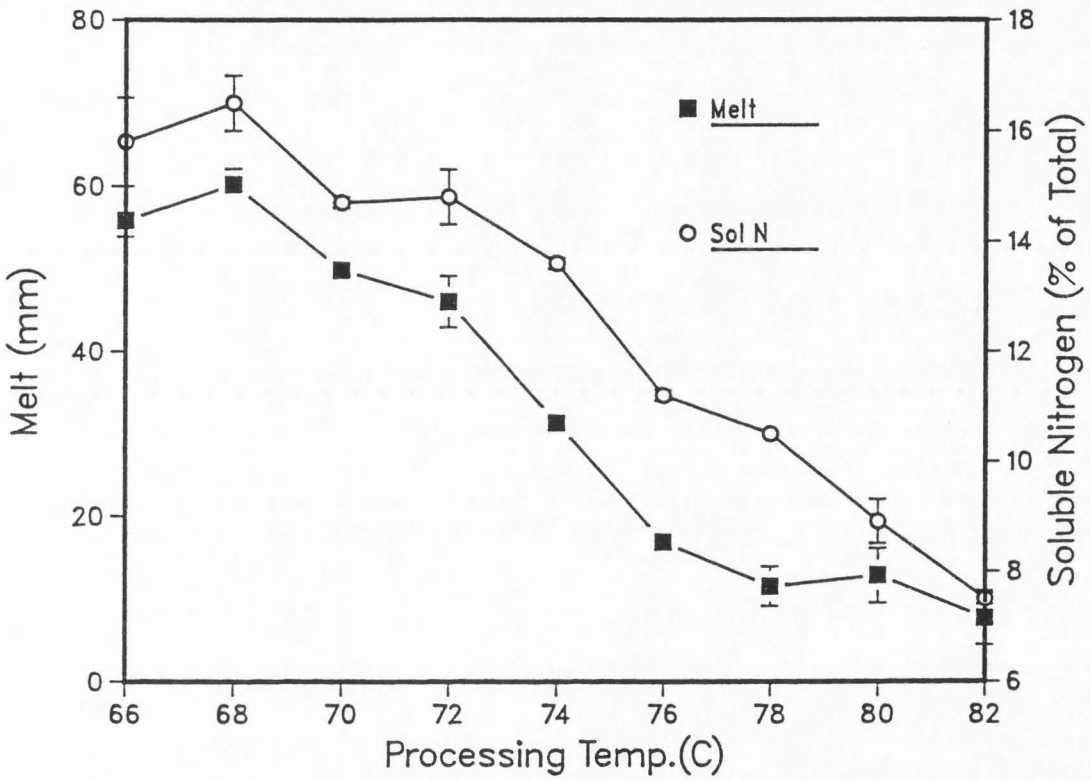


Figure 32. Effect of processing temperature on meltability and soluble nitrogen at pH 4.6.

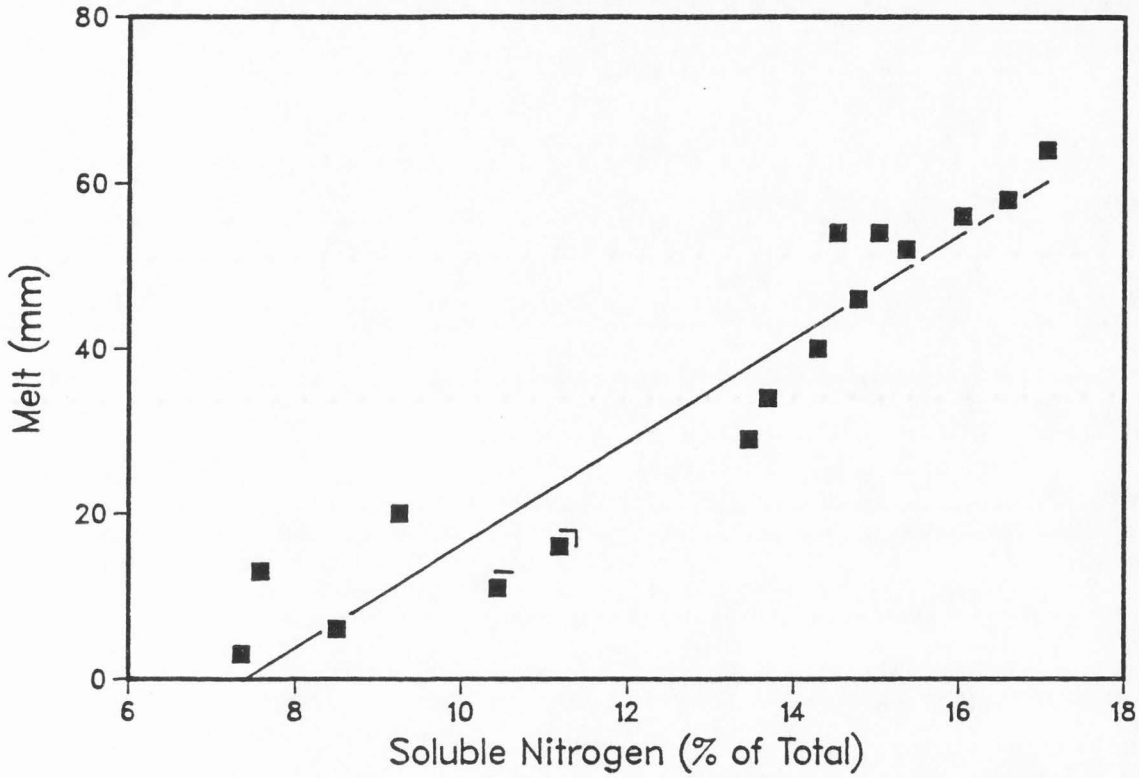


Figure 33. Relationship between meltability of pasteurized process cheese food and soluble nitrogen at pH 4.6.

(Figure 34). Both β-lactoglobulin and α-lactalbumin pass the UF membrane (7, 67). Since bovine serum albumin is larger than these proteins, more is retained by the UF membrane, and it may have an important role in the functional properties of milk concentrated by UF. Bovine serum albumin is high in disulfides and may be involved in disulfide interaction or exchange with other proteins.

Blends of Cheddar and UF Curd and Comparison of Melt Tests

Blends of UF curd and Cheddar cheese with final processing temperature of 68°C had better melt characteristic than when the final cook temperature was 72°C (Figure 35). Using process cheese food cooked at 68°C as final processing temperature, a comparison was made of the Olson and Price (64) and Schreiber melting tests (51). Correlation between the tests was low, with R=.62. Mean comparisons for the two tests are in Table 12. Meltability,when measured by the Olson and Price method, was linearly

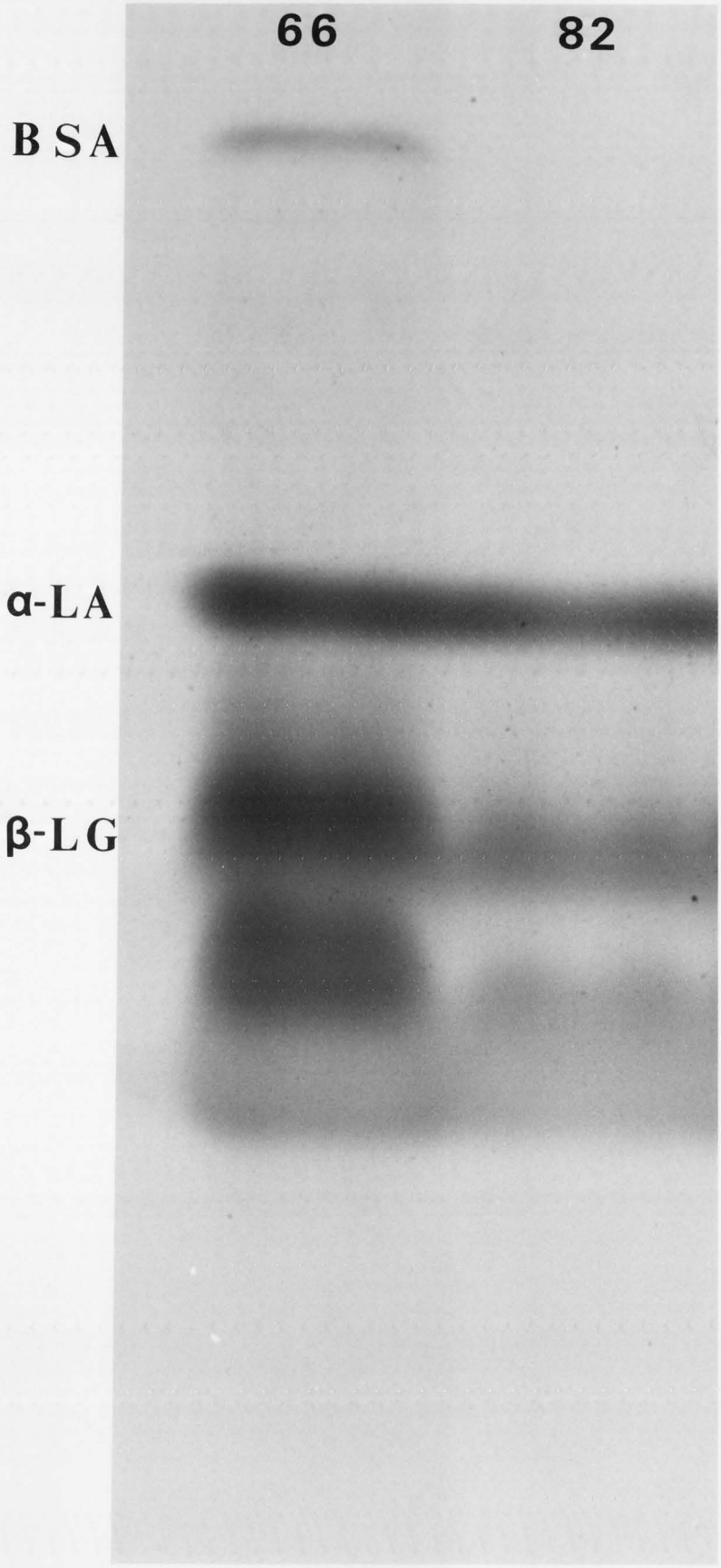
Table 12. Comparison of the Schreiber (51) and Olson and Price (64) melt tests for process cheese food made from blends of UF curd and Cheddar cheese.

%UF Curd	Schreiber (Score)	Olson Price (mm)
100	5.2^{b}	65^{a}
83	3.8^{a}	71^{ab}
66	5.0^{b}	79^{bc}
50	6.2^{cd}	86^{cd}
33	6.5^{cd}	89^{d}
16	5.7^{bc}	111^{e}
0	6.7^{d}	133^{f}

^{abcdef}Within columns, means followed by the same letter are not significantly different at α=.01 using Fishers protected LSD.

related to percent UF curd in the blend, R=.94. Results of the Schreiber test do not show such a clear relationship, R=.72. Although the Schreiber test uses a disk of cheese with

Figure 34. PAGE showing loss of β-lactoglobulin (β-lg) and bovine serum albumin (BSA) after cooking process cheese food to 66 or 82°C.


66
82
BSA
α-LA
β-LG

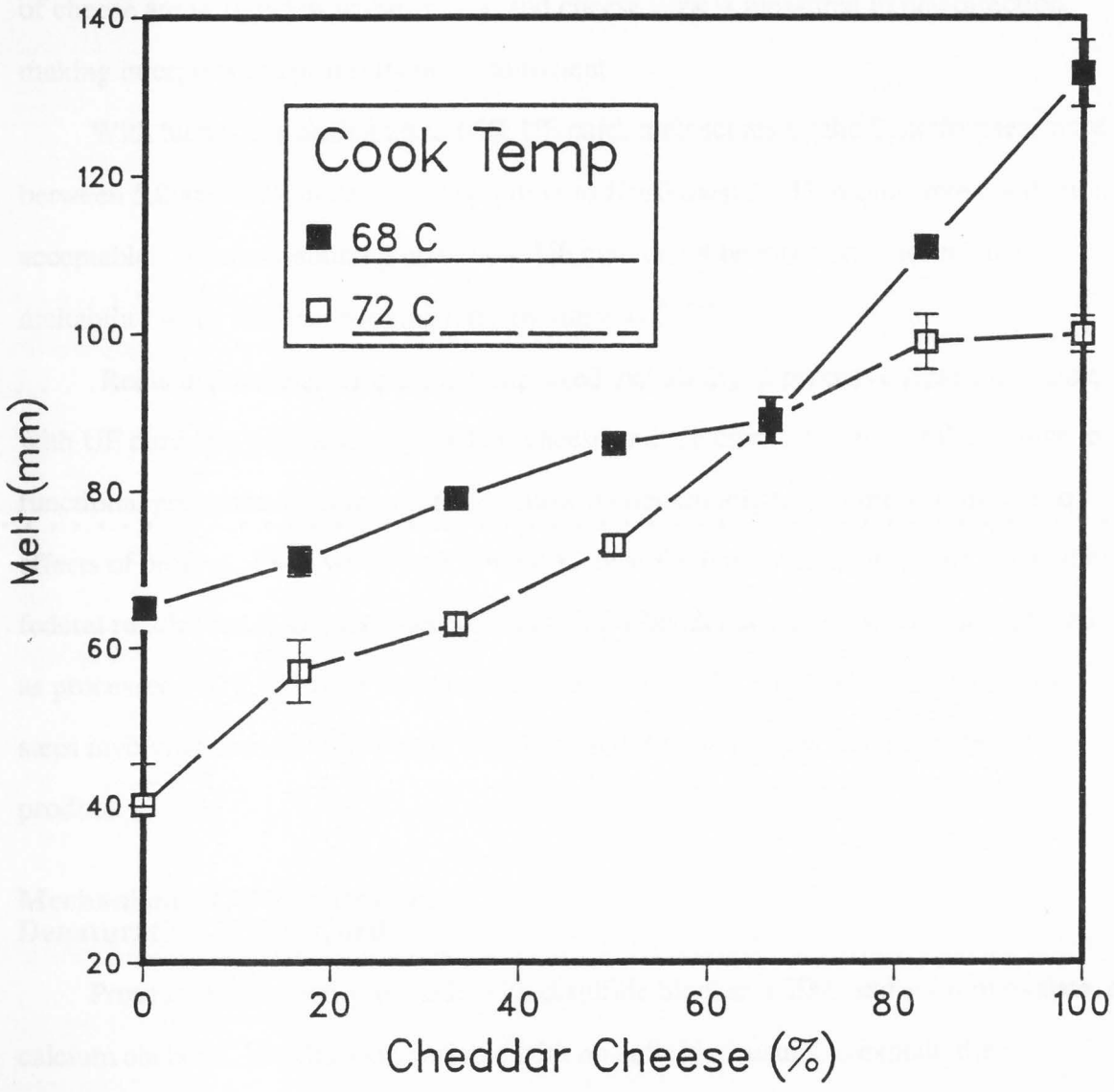


Figure 35. Meltability of pasteurized process cheese food made from blends of Cheddar cheese and UF curd. Cook temperatures are the final processing temperatures after initially heating Cheddar cheese to 82°C. Error bars represent standard error of the mean.

specified dimensions of 4.8 mm thick by 41 mm diameter, it was difficult to obtain slices with uniform thickness. The scoring method of placing an irregular shaped object on a grid of concentric circles may have contributed to some inconsistency. The Schreiber test is much faster to use and would be adequate for most quality control labs, but the Olson and Price method is more reliable for research. With the Olson and Price test, cylinders of cheese are weighed to within ± .1 g, and cheese flow is measured in one direction, making interpretation of results more consistent.

With blends containing up to 66% UF curd, melt scores by the Schreiber test were between 5.0 and 6.7 (Table 12). According to Kosikowski (51), scores above 4.0 are acceptable. Blends containing up to 66% UF curd could be made with acceptable meltability when the final cooking temperature was 68°C.

Reducing cooking temperature improved meltability of process cheese food made with UF curd or with blends of Cheddar cheese and UF curd. Because of the change in functional properties of whey proteins following denaturation, UF curd is sensitive to effects of heating. Even when heated to 68°C, near the minimum temperature allowed by federal regulations (14), processed UF curd (0% Cheddar cheese) does not melt as well as processed 100% Cheddar cheese (Figure 35). In a UF curd production plant, any steps involving heating milk should be minimized if meltability is desired in the final product.

Mechanism of Whey Protein Denaturation in UF Curd

Process cheese food was made with disulfide blocker, NEM, and sodium oxalate, a calcium chelator. Results were variable with no definitive results to explain the mechanism of whey protein denaturation. Further research could focus on understanding the chemical changes that lead to denaturing whey proteins and loss of meltability in process cheese food made with UF curd.

SUMMARY

Ultrafiltration has been studied extensively as a technique for improving cheese manufacturing. Central to the heart of this research has been the possibility of increasing cheese yield with a resulting increase in profit. When used for production of hard cheeses or cottage cheese, yield increases have been slight, although UF has been used profitably for production of soft cheese such as Camembert or Brie. Production of Cheddar cheese for processing by UF techniques has proven both feasible and profitable. Acceptance of UF has required research on process efficiency, nutrient recovery, and handling milk of poor bacteriological quality.

Ultrafiltration of milk can be an efficient and valuable tool for milk processing. When used for production of Cheddar cheese for processing, it is important to know how nutrients are affected by the UF process. Milk is an important dietary source of fat, protein, calcium, phosphorus, riboflavin, and vitamin B_{12}. Fat is completely retained by the UF membrane, and is 100% recovered in the curd. During traditional Cheddar cheese manufacture, 7-10% of the fat is lost in the whey (87). Between 88 and 100% of protein nitrogen is recovered in UF curd, also contributing to an increased yield since only about 76% of the protein is retained in traditional cheese manufacture (87). Protein quality is not decreased during UF, since amino acid concentration as g/100 g protein does not change between the milk and 5× concentrates. UF should enhance the quality of cheese protein since most of the whey proteins, known to have a higher protein efficiency ratio (PER) than casein (43), are retained in the curd. Vitamin B_{12} recovery was 62.9 to 65.1% following UF and diafiltration of milk that was preacidified to pH 5.8. Recovery ranged from 77 to 98% with unacidified, non-diafiltered milk (unpublished data). Process cheese food from UF curd made from unacidified milk contained slightly more vitamin B_{12} than traditional Cheddar cheese. During UF of milk, riboflavin is lost in permeate as it is in whey during normal cheese making. Because riboflavin washes out

during diafiltration, UF cheese curd contains about half as much riboflavin as traditional cheese curd. Minerals, Ca and P, are strongly associated with casein micelles and are retained in UF curd in nearly the same concentration as in Cheddar cheese, providing milk is not acidified before UF.

One area of concern to milk processors is the bacteriological quality of milk. It is possible to ultrafilter milk with high colony counts. With as many as 7.8×10^6 CFU/ml, UF proceeded normally with permeation rates equivalent to high quality milk. The milk did not cause membrane fouling or any other obvious processing difficulties. Free fatty acids were higher in retentate made from milk with the greatest number of bacteria, and it might make cheese with inferior flavor. Nutrient recovery was similar in retentate from milk with both high and low colony counts.

Process efficiency is important when diafiltration is used. Diafiltration is necessary to control the final amount of lactose left in the retentate. If too much lactose remains, the lactic cultures will decrease the pH to form an acid curd with poor manufacturing characteristics and poor flavor. Too little lactose will result in curd with too high pH, since lactic organisms quit growing when lactose is depleted. The optimum diafiltration plan would minimize time and water usage while retaining the nutrient quality of the retentate. Diafiltration after 70% of the initial milk weight was removed with water equal to 38.5% of the milk weight, produced the most efficient UF process with minimal time and water. After UF, the retentate reached the desired pH of 5.1 to 5.2 when incubated with lactic cultures. Nutrient recovery was not reduced by this diafiltration plan.

UF curd can be made with a yield 16-18% greater than traditional Cheddar cheese (29), with resulting increase in profits. For example, if a manufacturer is getting 100 kg cheese from 1000 kg milk, yield of UF curd would be 116 kg cheese. Currently, Cheddar cheese for manufacturing sells for $2.93 per kg. Price obtained from traditional Cheddar cheese would be $293.00 per 100 kg and $339.88 for 116 kg of UF curd, both made from 1000 kg milk. That would mean an increased income of $46.88 for every

1000 kg milk that passes through the plant. Clearly, the economic advantage is obvious for use of the UF process. Currently, some process cheese manufacturers use about 25% UF curd in the blend, with resulting increased income of $11.72 per 1000 kg milk.

Ultrafiltration can be used to produce Cheddar cheese for processing, called UF curd. This product is different from Cheddar cheese in both flavor and texture. Because of the inclusion of whey proteins in UF curd, it has different functional properties when used in process cheese and process cheese foods. When process cheese is made with 100% UF curd, it does not have the typical melting characteristics expected of process cheese. Many factors influence meltability of process cheese food made from UF curd. Apparently, acidifying milk before UF to remove calcium does not improve meltability. A 16 s heat treatment of milk to 61, 72, or 83°C before ultrafiltration does not significantly affect meltability. When retentate is subjected to a 16 s heat treatment after UF but before fermentation and being processed into UF curd, meltability of process cheese food is decreased with higher heat treatments. The most significant factor affecting meltability is the cooking temperature of process cheese food. Meltability decreases as cooking temperatures increases from 66 to 82°C because of whey protein denaturation and possible complexing with casein. When UF curd is cooked to temperatures typically used by cheese processors, 82°C, the process cheese food is very resistant to melting. A product with better meltability can be produced by processing UF curd to 65.6°C, meeting the legal minimum temperature for cheese processing. For process cheese manufacturers, use of UF curd requires some process modification. Higher temperatures kill more potential spoilage organisms and extend the shelf life of process cheese. Possibly, better sanitation practices could eliminate the necessity for higher cooking temperatures. Also, process cheese is less viscous at higher temperatures and will spread better on the casting belts used to form slices, probably the most popular form of cheese on the market. Cost of redesigning the casting belts to accommodate more

viscous products could be offset by profits realized from using more UF curd in the blend.

UF curd can be manufactured in a closed system, with no contamination from spoilage organisms. Shelf life of process cheese made from UF curd could be excellent even when processed at 65.6°C. The Cheddar cheese used in the blend would likely contain mold spores and undesirable bacteria, depending on its age and type of packaging. If a manufacturer wanted to increase usage of UF curd in a process cheese blend, it might be practical if the Cheddar cheese were first heated to normal processing temperatures of 82°C, cooled, UF curd added and then processed to a temperature barely above the legal minimum of 65.6°C for 30 s. When this method was used, blends of up to 66% UF curd produced process cheese food of acceptable meltability compared to commercial products. The net effect could be an increase in income of $31.25 per 1000 kg milk, when compared with using cheese produced by traditional methods.

Clearly, economic advantages of UF exist for producers of Cheddar cheese for processing. Because of different functional properties of UF curd, manufacturers must carefully control its use in process cheese food to achieve desirable products. Vitamins and minerals recovered during UF and production of UF curd is similar to recovery in Cheddar cheese. With understanding of its functional limitations, processors can harvest the financial benefits of UF curd, while providing products of excellent nutritional quality.

CONCLUSIONS

1. Amino acid composition of retentate (g/100 g protein) did not change during UF of milk to a 5× concentration.

2. Cheese curd made from ultrafiltered milk contained a higher percentage of protein and vitamin B_{12} but less fat, phosphorous, calcium and riboflavin than Cheddar cheese made from the same milk.

3. Ultrafiltration of milk with high bacterial counts proceeded normally.

4. Diafiltration after 70% volume reduction saved time and water without reducing nutrient recovery.

5. Riboflavin recovery was reduced when milk was heated to 72 or 82°C before UF. Riboflavin, calcium, and phosphorous recovery decreased when milk was acidified to pH 6.2 or 5.8 before UF.

6. The pH of milk before UF significantly affected meltability of pasteurized process cheese food made from UF curd. Process cheese food made from unacidified, ultrafiltered milk (pH 6.6) had significantly better meltability than when milk was acidified to pH 6.2 or 5.8. Cooking temperature of pasteurized process cheese food had an even greater effect on meltability. Process cheese food always melted better when cooked to 70°C than when cooked to 76 or 81°C.

7. Decreased meltability of pasteurized process cheese food made from UF curd cooked to high temperatures (82°C) was associated with increased whey protein denaturation. Heat causes denaturation of whey proteins, but a specific chemical mechanism was not found.

8. Pasteurized process cheese food made from blends of UF curd and Cheddar cheese had acceptable meltability with up to 66% UF curd. The cheese was made by first heating the Cheddar cheese to 82°C, cooling and then adding sodium citrate, water and UF curd, followed by a final cooking temperature of 68°C.

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APPENDIXES

Appendix A: Analysis of Variance of Amino Acid Concentration

Table 13. Analysis of variance of amino acids in milk during ultrafiltration to a 5× concentration.

	Mean Squares		
Amino Acid	Treatments	Reps	Error
Asp	.5819	.0250	1.4309
Glu	2.0673	.3626	.9914
Ser	.9088	1.0130	.4846
Gly	.0148	.0006	.0106
His	.0758	.0233	.0458
Arg	.2662	.5261	.1000
Thr	.3497	.2778	.1286
Ala	.0459	.0856	.0288
Pro	.1055	.0316	.2622
Tyr	.2465	.3040	.7503
Val	.0762	.0455	.7635
Met*	.2011	.0337	.0361
Ile	.0572	.0026	.1953
Leu	.3072	.0732	.6343
Phe	.1639	.5249	.0721
Lys*	4.6796	7.7699	.6631

For each ANOVA, there were four degrees of freedom (df) for treatments, two df for replications, eight df for error, and fourteen df total.

*Indicates significant difference between treatments at $\alpha = .05$ level.

Table 14. Analysis of variance of amino acids in UF pasteurized process cheese food and Cheddar cheese.

Amino Acid	Mean Squares	
	Treatments	Error
Asp**	.2184	.0133
Glu	.4299	.1825
Ser	.0541	.0075
Gly	.0004	.0012
His	.0553	.0079
Arg	.1805	.0176
Thr	.1346	.0196
Ala	.0193	.0144
Pro	.1589	.1183
Tyr	.2006	.0201
Val	.0106	.0472
Met	.2021	.1153
Ile	.0369	.0104
Leu	.0076	.0303
Phe	.1768	.0626
Lys**	1.6669	.0189

For each ANOVA, there were three degrees of freedom (df) for treatments, four df for error, and seven df total.

**Indicates significant difference between treatments at $\alpha = .01$ level.

Appendix B: Analysis of Variance of Nutrient Composition and Recovery

Table 15. Analysis of variance of nutrient composition of 5× retentate after ultrafiltration of milk with high bacterial numbers.

Constituents	Mean Squares	
	Treatments	Error
Solids**	36.1813	.0015
Protein**	2.9758	.0068
Free Fatty Acids**	1.5286	.0007
Nonprotein Nitrogen**	5.4159	.0581
Lactose**	.3337	.0024

For each ANOVA, there were two degrees of freedom (df) for treatments, three df for error, and five df total, except lactose had six df for error and eight df total.

**Indicates significant difference between treatments at $\alpha = .01$ level.

Table 16. Analysis of variance of nutrient composition of 5× retentate after varying the beginning point of diafiltration.

Constituents	Mean Squares	
	Treatments	Error
Solids**	.8092	4.445×10^{-4}
Protein**	.2917	1.934×10^{-3}
Fat**	.4174	1.696×10^{-3}
Rennet Clottable Nitrogen	8.656	4.049
Lactose**	.1308	4.244×10^{-3}
Ionic Calcium**	1.734×10^{-4}	2.1×10^{-7}
Riboflavin	1.295×10^{-4}	1.708×10^{-4}
Calcium**	3.874×10^{-4}	3.182×10^{-5}
Phosphorus	2.155×10^{-5}	5.92×10^{-6}
Buffer Capacity	.0562	.0241

For each ANOVA, there were two degrees of freedom (df) for treatments, three df for error, and five df total, except Ca, P, buffer capacity, and lactose had six df for error and eight df total.

**Indicates significant difference between treatments at $\alpha = .01$ level.

Table 17. Analysis of variance of solids recovery following preheating and acidification of milk before UF.

Source	df	MS	F value	Significant α
Rep	1	2.832	.93	.3635
Preheat	2	3.517	1.15	.3631
pH	2	4.468	1.46	.2870
Heat × pH	4	.3558	.12	.9728
Error	8	3.050		
Corrected Total	17			

Table 18. Analysis of variance of protein recovery following preheating and acidification of milk before UF.

Source	df	MS	F value	Significant α
Rep	1	.2006	.03	.8676
Preheat	2	9.532	1.41	.2992
pH	2	4.527	.67	.5388
Heat × pH	4	4.521	.67	.6320
Error	8	6.768		
Corrected Total	17			

Table 19. Analysis of variance of phosphorus recovery following preheating and acidification of milk before UF.

Source	df	MS	F value	Significant α
Rep	1	1.614	.44	.5276
Preheat	2	15.46	4.18	.0573
pH	2	252.2	68.14	.0001
Heat × pH	4	.9858	.27	.8916
Error	8	3.701		
Corrected Total	17			

Table 20. Analysis of variance of calcium recovery following preheating and acidification of milk before UF.

Source	df	MS	F value	Significant α
Rep	1	917.1	52.81	.0001
Preheat	2	102.2	5.89	.0268
pH	2	613.4	35.33	.0001
Heat × pH	4	19.51	1.12	.4099
Error	8	17.36		
Corrected Total	17			

Table 21. Analysis of variance of lactose recovery following preheating and acidification of milk before UF.

Source	df	MS	F value	Significant α
Preheat	2	.8582	1.68	.2948
pH	2	1.519	2.98	.1612
Error	4	.5097		
Corrected Total	8			

Table 22. Analysis of variance of riboflavin recovery following preheating and acidification of milk before UF.

Source	df	MS	F value	Significant α
Preheat	2	5.165	27.44	.0046
pH	2	9.000	47.82	.0016
Error	4	.1881		
Corrected Total	8			

Appendix C: Analysis of Variance of Process Cheese Food Meltability

Table 23. Analysis of variance of Percent Cheddar cheese with meltability measured by the Schreiber (51) test.

Source	df	Mean Square	F value	Significant α
Percent Cheddar	6	2.968	22.67	.0001
Error	14	.1310		
Corrected Total	20			

Table 24. Analysis of variance of Percent Cheddar cheese with meltability as measured by the Olson and Price (64) test.

Source	df	Mean Square	F value	Significant α
Percent Cheddar	6	1713	118.4	.0001
Error	14	14.48		
Corrected Total	20			

Appendix D: Regression Analysis for Soluble Nitrogen and Meltability and Comparison of Olson and Price and Schreiber Melt Tests.

Table 25. Regression analysis of meltability as tested by the Olson and Price (64) method and percent Cheddar cheese in pasteurized process cheese food.

Source	df	MS	F value	Significant α
Percent Cheddar	1	9331.5	152.67	.0001
Error	19	1152.8		
Corrected Total	20			

Melt = .6319 × Percent Cheddar + 59.394 R^2 = .890

Table 26. Regression analysis of meltability as tested by the Schreiber (51) method and percent Cheddar cheese in pasteurized process cheese food.

Source	df	MS	F value	Significant α
Percent Cheddar	1	10.08	20.04	.0003
Error	19	.5031		
Corrected Total	20			

Melt = .0208 × Percent Cheddar + 4.539 R^2 = .513

Table 27. Regression analysis of meltability versus soluble nitrogen in pasteurized process cheese food.

Source	df	MS	F value	Significant α
Soluble Nitrogen	1	6535.2	144.97	.0001
Error	16	45.08		
Corrected Total	17			

Melt = 6.244 × Soluble Nitrogen - 46.221 R^2 = .9006

Table 28. Regression analysis of meltability tested by the Olson and Price (64) method versus the Schreiber (51) method in pasteurized process cheese food.

Source	df	MS	F value	Significant α
Schreiber Score	1	4013.7	11.78	.0028
Error	19	340.6		
Corrected Total	20			

Melt (Olson and Price) = 14.2945 × Melt (Schreiber) + 11.1685 R^2 = .383

Appendix E: Interaction Between Cooking Temperature and Preheat Temperature

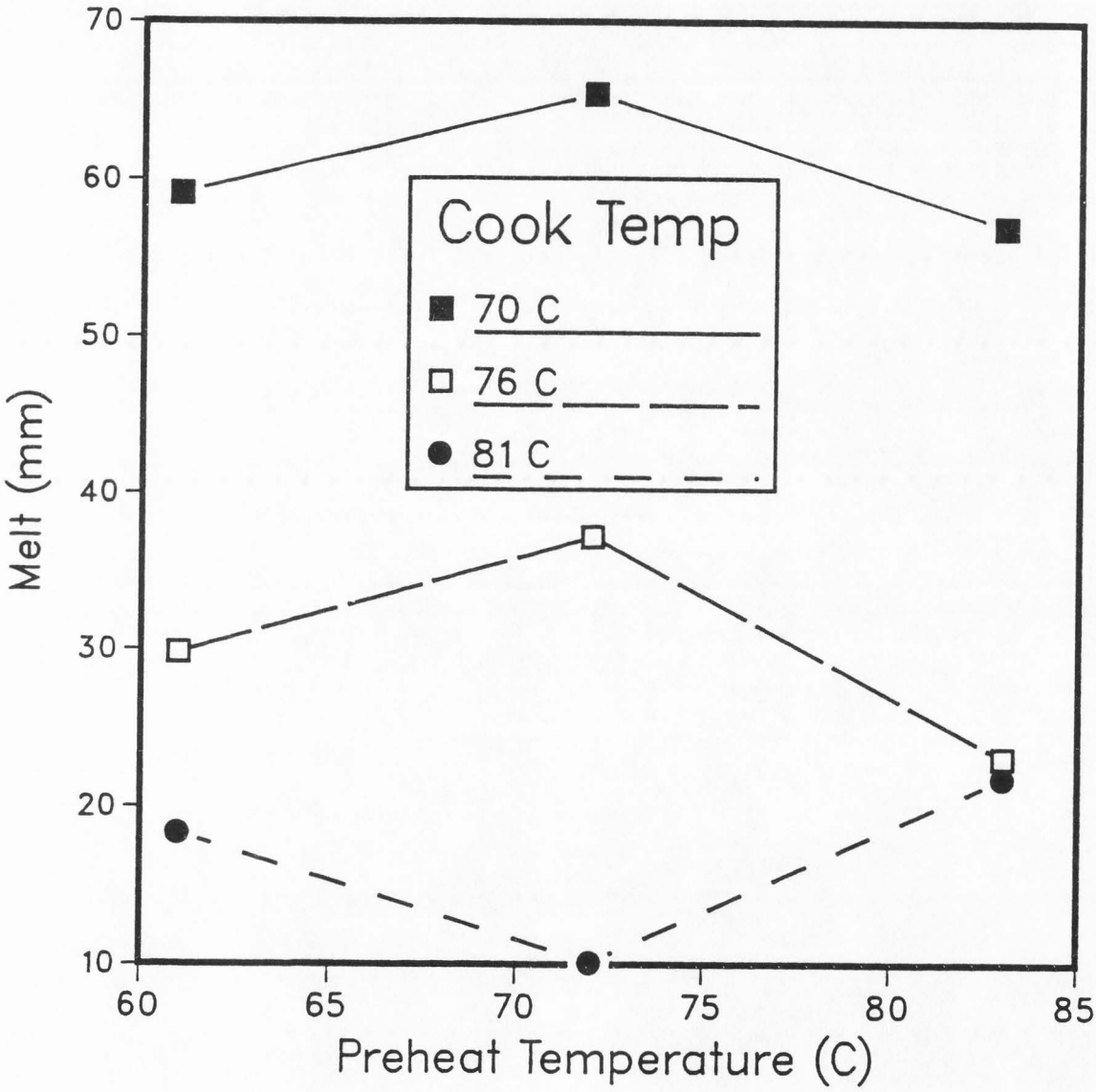

Figure 36. Significant interaction of cooking temperature with preheat temperature on process cheese food meltability.

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